

NOVEL NITROPHENYL MUSTARD AND NITROPHENYLAZIRIDINE
ALCOHOLS AND THEIR CORRESPONDING PHOSPHATES AND THEIR USE
AS TARGETED CYTOTOXIC AGENTS

5 The present invention relates to novel nitrophenyl mustard and nitrophenylaziridine alcohols, to their corresponding phosphates, to their use as targeted cytotoxic agents; as bioreductive drugs in hypoxic tumours, and to their use in cell ablation, including gene-directed enzyme-prodrug therapy (GDEPT) and antibody-directed enzyme-prodrug therapy (ADEPT), in conjunction with nitroreductase enzymes.

10

Background to the invention

The use of tumour-selective prodrugs (relatively inactive compounds that can be selectively converted to more active compounds *in vivo*) is a valuable concept in cancer therapy (see,
15 for example Denny, *Eur. J. Med. Chem.* (2001) 36, 577).

For example a prodrug may be converted into an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated antigen. The combination of such a prodrug with such an enzyme monoclonal/antibody
20 conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT), is disclosed in W088/07378.

A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT)
25 has been proposed as a method for treating tumour cells in patients using prodrugs.

Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug within the tumour cells
30 (Huber et al., *Proc. Natl. Acad. Sci. USA* (1991) 88, 8039). Alternatively, non-viral methods for the delivery of genes have been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-

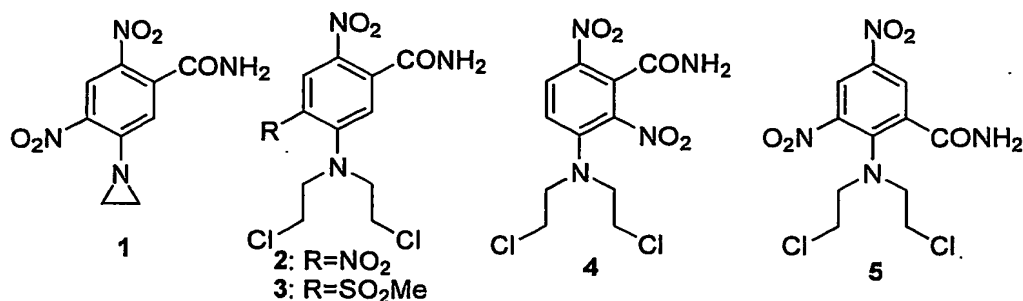
mediated DNA transfer. These are reviewed in Morgan & French, *Annu. Rev. Biochem.*, 1993, 62; 191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems (Denny et al US 6,310,237).

- 5 4-Nitroaromatic compounds are reduced by both mammalian and bacterial flavoprotein enzymes, which effect stepwise addition of up to six electrons. The major enzymatic metabolite is usually the 4-electron reduced species (hydroxylamine).

- A number of nitrophenyl mustards and nitrophenylaziridines have been reported as
10 prodrugs for use in gene-directed enzyme-prodrug therapy (GDEPT) in conjunction with nitroreductase enzymes. In particular, CB 1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide; (1) [shown below] is reported to be a substrate for the aerobic nitroreductase NTR (nfsB gene product) isolated from *E. coli* B (Boland et al., *Biochem. Pharmacol.* 1991, 41, 867-875; Anlezark et al., *Biochem. Pharmacol.*
15 1992, 44, 2289-2295; Parkinson et al., *J. Med. Chem.* 2000, 43, 3624). This compound has been used as a prodrug in both ADEPT (Knox et al., *Biochem. Pharmacol.*, 1995, 49, 1641-1647) and GDEPT (Bridgewater et al., *Eur. J. Cancer*, 1995, 31A, 2362-2370; Bailey et al., *Gene Ther.*, 1996, 3, 1143-1150; Bailey and Hart, *Gene Ther.*, 1997, 4, 80-81; Green et al., *Cancer Gene Ther.*, 1997, 4, 229-238)
20 applications, including a clinical trial (Chung-Faye et al., *Clin. Cancer Res.*, 2001, 7, 2662-2668).

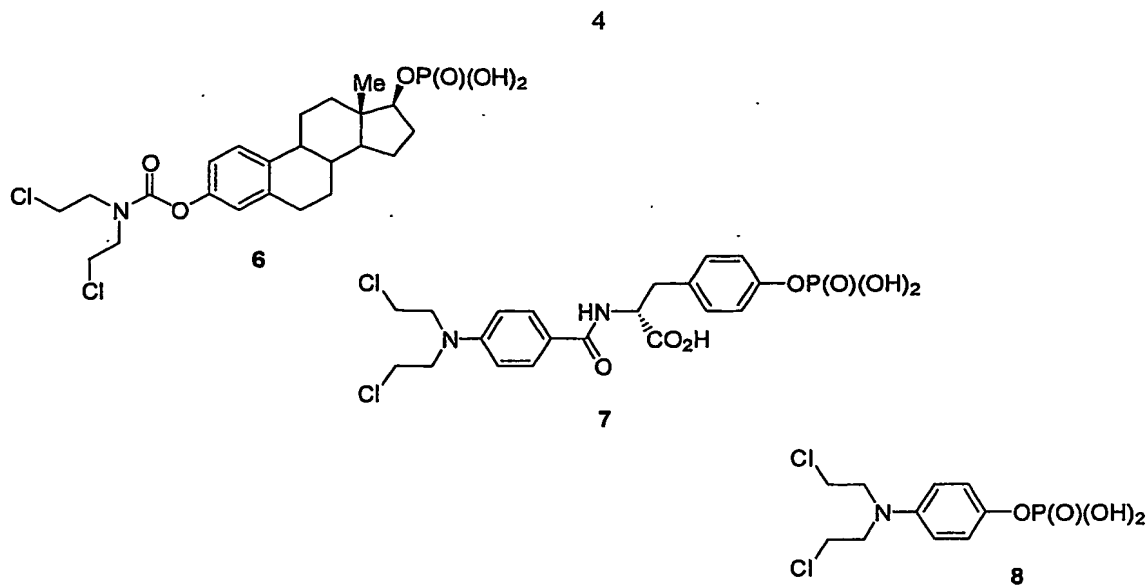
- Similarly, the dinitrophenyl mustard SN 23862 (2) is also a substrate for NTR, and shows selective toxicity towards cell lines that express the enzyme. It is activated by nitro group
25 reduction (Palmer et al., *J. Med. Chem.*, 1995, 38, 1229; Kestell et al., *Cancer Chemother. Pharmacol.*, 2000, 46, 365-374). The 4-SO₂Me derivative (3) was also a substrate (Atwell et al., *Anti-Cancer Drug Des.*, 1996, 11, 553), as were the regioisomers (4) and (5) (Friedlos et al., *J. Med. Chem.*, 1997, 40, 1270).

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However, compounds of this type were not very effective as bioreductive prodrugs when these compounds were activated in hypoxic tumour tissue by endogenous reductase enzymes, showing potency ratios of 2-5 fold under hypoxic conditions relative to oxic conditions in the wild-type AA8 cell line, using a clonogenic assay (Palmer et al., J. Med. Chem. 1996, 39, 2518-2528).

Some phosphate analogues of mustards have been described, for the purpose of solubilising the compounds. The best known is estramustine phosphate (Estracyt; 6), which has been shown to bind to tubulin binding domains on various microtubule-associated proteins (Moraga et al., Biochim. Biophys. Acta, 1992, 1121, 97-103), and which has been shown to be active in advanced breast cancer (Keren-Rosenberg et al., Semin. Oncol., 1997, 24 (Suppl. 3), 26-29), but has not been shown to be activated by NTR or hypoxia. Another study has also shown estramustine phosphate to be a radiation sensitizer (Kim et al., Int. J. Radiat. Oncol. Biol. Phys., 1994, 29, 555-557). The phenol mustard phosphate analogue 7 is a carboxypeptidase substrate that is not activated under hypoxic conditions, (Matsui et al., Japanese Patent 07082280 A2, 1995), and the solubilised mustard 8 has been described as a phosphatase inhibitor but has not been shown to be activated under hypoxic conditions (Workman, Chem.-Biol. Interact., 1978, 20, 103-112).

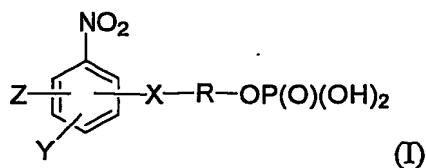


It is an object of the present invention to provide a specific class of nitrophenyl mustards
 5 and aziridines, bearing short-chain alcohols, and their corresponding phosphates for use as
 targeted cytotoxic agents or bioreductive prodrugs or to at least provide the public with a
 useful alternative.

Summary and detailed description

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In a first aspect, the present invention provides novel phosphate compounds of Formula 1



wherein:

- 15 X represents at any available ring position $-\text{CONH}-$, $-\text{SO}_2\text{NH}-$, $-\text{O}-$, $-\text{CH}_2-$, $-\text{NHCO}-$ or $-\text{NHSO}_2-$;

R represents a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

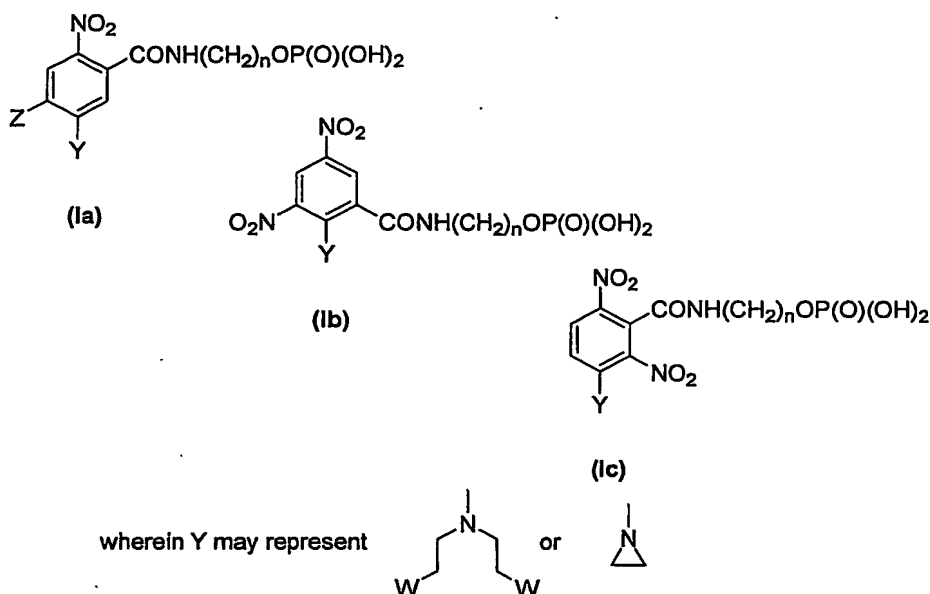
- 20 Y represents at any available ring position $-\text{N-aziridinyl}$, $-\text{N}(\text{CH}_2\text{CH}_2\text{W})_2$ or $-\text{N}(\text{CH}_2\text{CHMeW})_2$, where each W is independently selected from halogen or $-\text{OSO}_2\text{Me}$.

Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

and pharmaceutically acceptable salts and derivatives thereof.

5

In a preferred embodiment, the phosphate compound of Formula (I) is selected from a compound represented by formulae (Ia), (Ib) or (Ic)



10 and wherein

n represents 1 to 6

Z represents -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

where each W is independently selected from halogen or -OSO₂Me

and pharmaceutically acceptable salts and derivatives thereof.

15

Preferably, the phosphate compound of Formula (I) is selected from the following:

2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

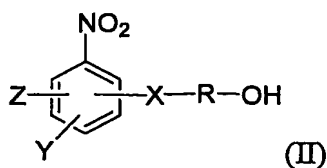
3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

20 2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

- 2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl}amino)ethyl dihydrogen phosphate;
 2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl
 methanesulfonate;
 2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate ;
 5 2-[(2-Iodoethyl)-2,4-dinitro-6-({[2-(phosphonooxy)ethyl]amino}carbonyl)-anilino]ethyl
 methanesulfonate;
 2-[(2-Chloroethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-
 carbonyl]anilino]ethyl methanesulfonate;
 3-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl}amino)propyl dihydrogen phosphate;
 10 2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl
 methanesulfonate;
 2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-
 carbonyl]anilino]ethyl methanesulfonate; and
 2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl
 15 methanesulfonate.

In a second aspect, the present invention provides alcohol compounds of Formula (II)



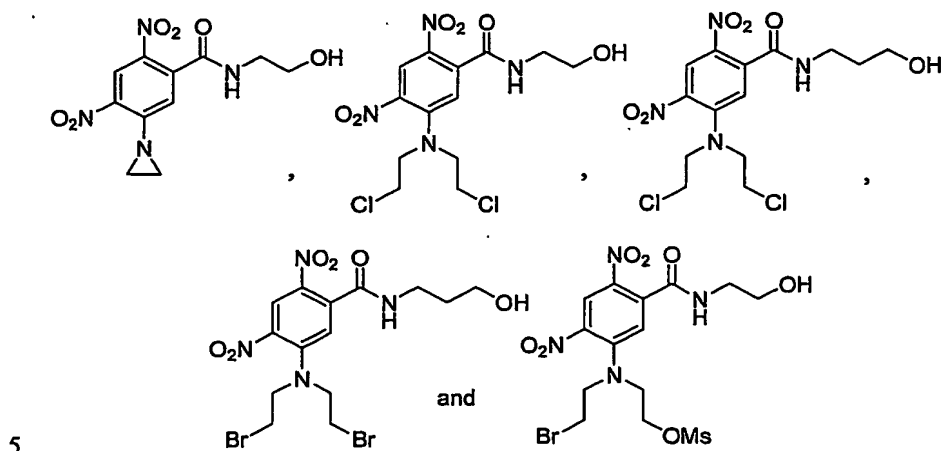
20 wherein:

X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y represents at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂, or
 25 -N(CH₂CHMeW)₂ where each W is independently selected from halogen or -OSO₂Me;

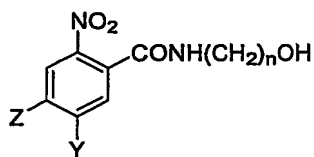
Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, with the proviso that

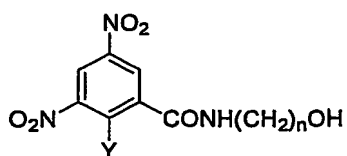


are excluded.

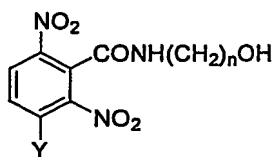
In a preferred embodiment, the alcohol compound of Formula (II) is selected from a
10 compound represented by formulae (IIa), (IIb) or (IIc)



(IIa)

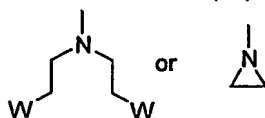


(IIb)



(IIc)

wherein Y may represent



or

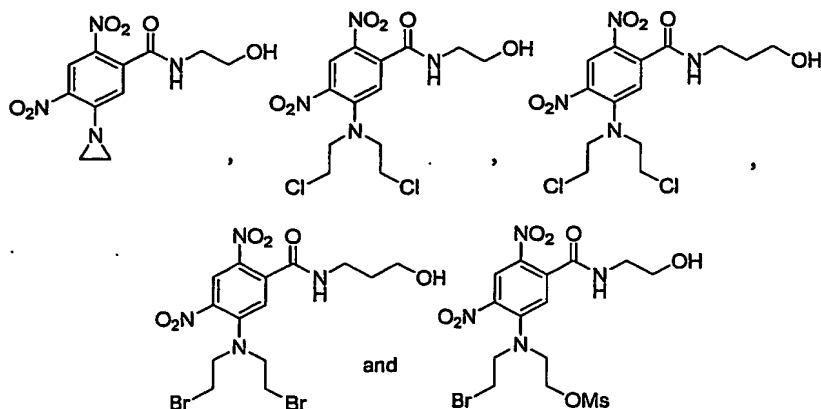
and wherein

n represents 1 to 6

Z represents -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

where each W is independently selected from halogen or -OSO₂Me

and pharmaceutically acceptable salts and derivatives thereof with the proviso that



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are excluded.

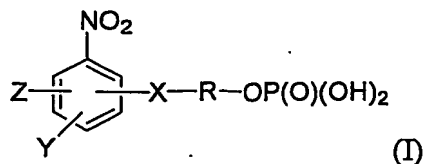
Preferably the compound of Formula (II) is selected from the following:

- N-(3-Hydroxypropyl)-5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzamide;
- 10 N-(3-Hydroxypropyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;
- 15 5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methanesulfonyl)-2-nitrobenzamide;
- 2[(2-Bromoethyl)-5-[[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate;
- 5-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2,4-dinitrobenzamide;
- 2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
- 20 2-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
- 2-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;
- 25 2-[Bis(2-chloroethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;

- 2-[Bis(2-bromoethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;
 2-[Bis(2-chloroethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;
 2-[Bis(2-bromoethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;
 2-[Bis(2-bromopropyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
 5 2-((2-Bromoethyl)-2-{[(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl
 methanesulfonate;
 2-((2-Bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl
 methanesulfonate;
 2-((2-Chloroethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl
 10 methanesulfonate;
 2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
 2-((2-Iodoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl
 methanesulfonate;
 3-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-2,6-dinitrobenzamide;
 15 2-((2-Bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,4-dinitroanilino)ethyl
 methanesulfonate;
 3-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-2,6-dinitrobenzamide;
 2-((2-bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl
 methanesulfonate;
 20 3-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-2,6-dinitrobenzamide;
 2-((2-Bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,4-dinitroanilino)ethyl
 methanesulfonate;
 2-((2-Chloroethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl
 methanesulfonate; and
 25 2-((2-Iodoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl
 methanesulfonate.

In a third aspect of the invention there is provided a method of preparing the phosphates represented by the general formula (I);

10



wherein:

X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or
 5 -NHSO₂-;

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

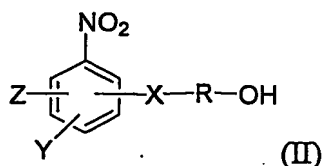
Y represents at any available ring position -N-aziridiny1 or -N(CH₂CH₂W)₂, where each
 10 W is independently selected from halogen or -OSO₂Me;

Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

and pharmaceutically acceptable salts and derivatives thereof;

15 the method including the step of

(i) phosphorylating a compound of formula (II)



20 wherein:

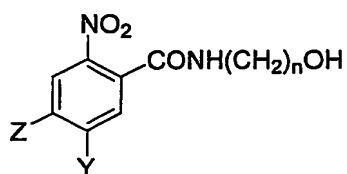
X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or
 -NHSO₂-;

Y represents at any available ring position -N-aziridiny1, -N(CH₂CH₂W)₂, or -N(CH₂CH
 25 MeW)₂ where each W is independently selected from halogen or -OSO₂Me;

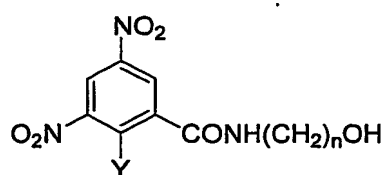
Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom.

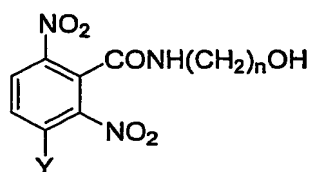
In a fourth aspect there is provided a method of preparing a compound of formulae (IIa),
 5 (IIb) or (IIc)



(IIa)

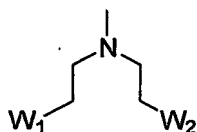


(IIb)



(IIc)

wherein Y may represent



and wherein

10 n represents 1 to 6

Z represents -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

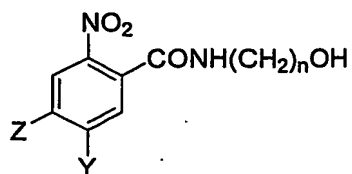
where W₁ is halogen and W₂ is -OSO₂Me

and pharmaceutically acceptable salts and derivatives thereof;

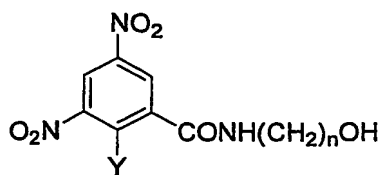
15 the method including the step of

reacting a compound of formulae (IIa'), (IIb') or (IIc') optionally with heating

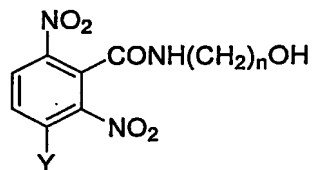
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(IIa')

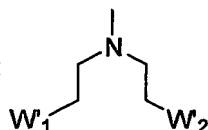


(IIb')



(IIc')

wherein Y may represent



wherein W'₁ and W'₂ are each halogen;

- 5 with an effective amount of silver methanesulfonate (AgOMs) in a solvent to give a compound of formulae (IIa), (IIb) or (IIc) defined above.

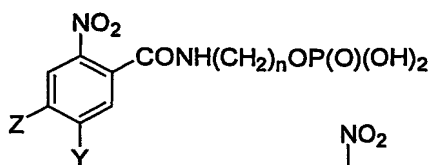
It is to be appreciated that in the method defined immediately above where W'₁ and W'₂ are either iodine and/or bromine that the iodine and/or bromine can be partially or
 10 completely substituted with -OSO₂Me. In the situation where either or both of W'₁ and W'₂ represent chlorine, the chlorine is inert and cannot be substituted with -OSO₂Me.

Preferably the solvent is selected from MeCN or other polar non-protic solvent.

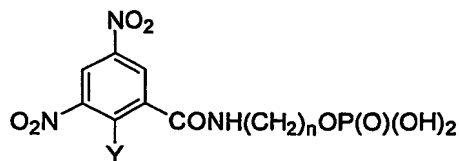
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In a fifth aspect there is provided a method of preparing a compound of formulae (Ia), (Ib) or (Ic)

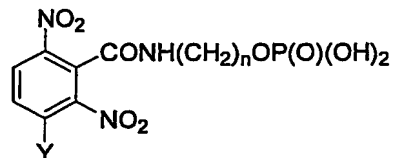
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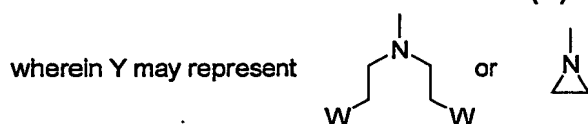
(Ia)



(Ib)



(Ic)



and wherein

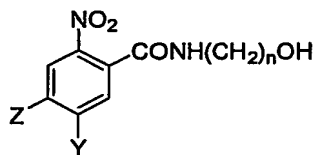
n represents 1 to 6

Z represents -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

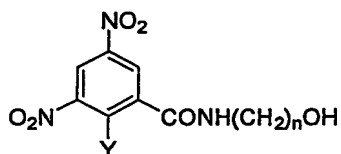
- 5 where each W is independently selected from halogen or -OSO₂Me
and pharmaceutically acceptable salts and derivatives thereof

the method including the step of

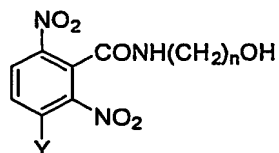
phosphorylating a compound represented by formulae (IIa), (IIb) or (IIc)



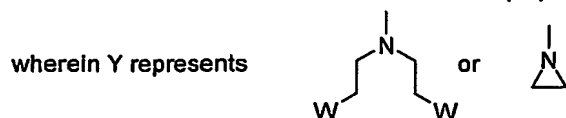
(IIa)



(IIb)



(IIc)



10

and wherein

n represents 1 to 6

Z represents -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

where each W is independently selected from halogen or -OSO₂Me

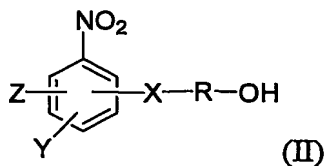
5 and pharmaceutically acceptable salts and derivatives.

In a sixth aspect there is provided a compound of formula (I), formula (Ia), (Ib) or (Ic) or formula (IIa), (IIb) or (IIc) obtained by any one of the preparative methods defined above.

10 In a seventh aspect, the present invention provides a method for the use as prodrugs suitable for

(i) GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (anti-body directed enzyme prodrug therapy) in conjunction with at least one nitroreductase enzyme; or

15 (ii) Use as one or more hypoxia-selective cytotoxins, including the step of administering a compound of Formula (I) as defined above or a compound of Formula (II)



wherein:

20 X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y represents at any available ring position -N-aziridiny, -N(CH₂CH₂W)₂, or -N(CH₂CH MeW)₂ where each W is independently selected from halogen or -OSO₂Me;

25

Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and

30 pharmaceutically acceptable salts and derivatives thereof;

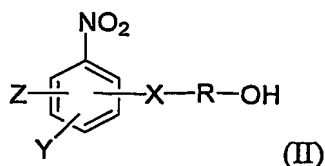
or a mixture thereof in a therapeutically effective amount to tumour cells in a subject.

Preferably, the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. coli* or by orthologous genes in *Clostridia* species.

5

Preferably the method includes the further step of irradiating the tumour cells.

In an eighth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-directed enzyme-prodrug therapy) or ADEPT (antibody-
 10 directed enzyme prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent including the step of administering a compound of Formula (I) as defined above or a compound of Formula (II)



15 wherein:

X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y represents at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂ or -N(CH₂CH
 20 MeW)₂, where each W is independently selected from halogen or -OSO₂Me;

Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including
 25 hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof;
 or a mixture thereof in a therapeutically effective amount to target tumour cells in a subject.

Preferably the nitroreductase enzyme is encoded for by the *nfsB* gene of *E. coli* or by orthologous genes in *Clostridia* species.

Preferably the method includes the further step of irradiating the tumour cells.

5

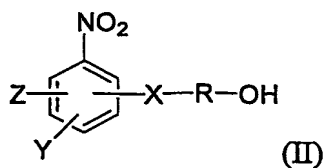
It is to be appreciated that with ADEPT it may be necessary to supply a reducing co-factor, because these may not be present in significant concentrations outside cells.

It is envisaged that a synthetic co-factor could be used to stimulate activation of the pro-drug by the likes of an intracellular enzyme. The same issue does not arise with

10 GDEPT because there are several intracellular reducing co-factors such as the likes of NADH and NADPH in significant concentrations.

In a ninth aspect of the present invention, there is provided a method of cell ablation utilising at least one nitroreductase enzyme, wherein the method includes the step of

15 administering a compound of Formula (I) as defined a above or a compound of Formula (II)



wherein:

20 X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y represents at any available ring position -N-aziridiny, -N(CH₂CH₂W)₂ or -N(CH₂CH MeW)₂, where each W is independently selected from halogen or -OSO₂Me;

25

Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides

30 therefrom; and pharmaceutically acceptable salts and derivatives thereof,

or a mixture thereof in an effective amount to ablate cells, wherein said cells express at least one nitroreductase enzyme.

Preferably the nitroreductase enzyme is encoded for by the *nfsB* gene in *E. coli* or by
5 orthologous genes in *Clostridia* species.

Preferably, the cells that are targeted for ablation are tumor cells in tissue in a subject.

Preferably, the method of cell ablation utilising at least one nitroreductase enzyme is
10 delivered by either ADEPT or GDEPT technology.

Preferably, the cell ablation provides a substantially minimal bystander effect.

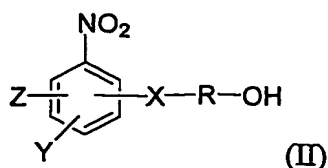
In a tenth aspect, the present invention provides a method of providing anticancer
15 therapy, wherein a compound of Formula (I) as defined above is administered in a therapeutically effective amount to tumour cells in a subject.

Preferably the therapeutically effective amount of said method is between about 20% to 100% of the maximum tolerated dose of said subject.

20

Preferably, the method includes the further step of irradiating the tumour cells.

In an eleventh aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of Formula (I) or
25 a compound of Formula (II)



wherein:

X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or
30 -NHSO₂-;

Y represents at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂ or -N(CH₂CH MeW)₂, where each W is independently selected from halogen or -OSO₂Me;

5 Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof,

10 or a mixture thereof, and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

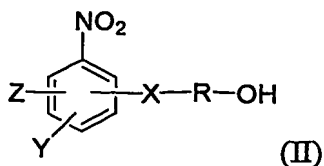
The pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser should preferably be non-toxic and should not interfere with the efficacy of the active ingredient.

15 The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, such as cutaneous, subcutaneous, or intravenous. It is to be appreciated that these factors could be readily determined by someone skilled in the art without undue experimentation.

20 Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene
25 glycol may be included. A capsule may comprise a solid carrier such as gelatin.

For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has a suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to
30 prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers antioxidants and/or other additives may be included as required.

In a twelfth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula (I) as defined above or a compound of Formula (II)



wherein:

X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

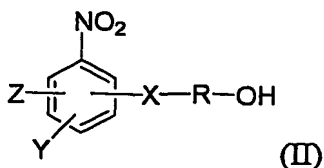
10 Y represents at any available ring position -N-aziridiny, -N(CH₂CH₂W)₂ or -N(CH₂CH MeW)₂, where each W is independently selected from halogen or -OSO₂Me;

Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

15 R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or mixtures thereof, for use in (i) GDEPT or (ii) as a hypoxia selective cytotoxin, to target cancer cells in a subject in need thereof.

20

In a thirteenth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula (I) as defined above or a compound of Formula (II)



wherein:

X represents at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y represents at any available ring position -N-aziridiny1, -N(CH₂CH₂W)₂ or -N(CH₂CH MeW)₂, where each W is independently selected from halogen or -OSO₂Me;

5 Z represents at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R represents a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof,

10 or mixtures thereof for use in cell ablation therapy to target cancer cells in a subject in need thereof.

While the compounds of the present invention will typically be used to target tumour cells or tumour tissues in human subjects, they may be used to target tumour cells or
15 tissues in other warm blooded animal subjects such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

As used throughout the specification the term "therapeutically effective amount", is to be understood as an amount of a compound of Formula (I) or Formula (II) as
20 defined above or a compound of any one of compounds Ia-Ic, or IIa-IIc as defined above or a mixture thereof that is sufficient to show benefit to a subject with cancer cells. The actual amount, rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment is within the responsibility of general practitioners and other medical doctors.

25

As used throughout the specification the term "halogen" includes chlorine, bromine or iodine.

It is to be understood that the compounds of the invention as defined above may be
30 administered alone or in combination with other treatments, especially radiotherapy and cytotoxic chemotherapeutic drugs, either simultaneously or sequentially dependent upon the condition to be treated.

As used throughout the specification the pharmaceutically acceptable derivatives and salts thereof include acid derived salts formed from are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic acids and the like and base derived salts formed from sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine and the like.

As used throughout the specification, the term cell ablation is to be understood as the killing of cells, that have been engineered to express an enzyme, such as a nitroreductase, by administration of a prodrug that is activated by that enzyme. As a result, cell ablation can be used to selectively ablate specified target cells or tissue through specific enzymatic expression of a nitroreductase for example, that is specifically expressed by the tissue and which can then be employed to activate a prodrug into an active metabolite to ablate the specified target cells or tissue. (Gusterson *et al. Endocrine Related Cancer*, 1997, 4, 67-74.)

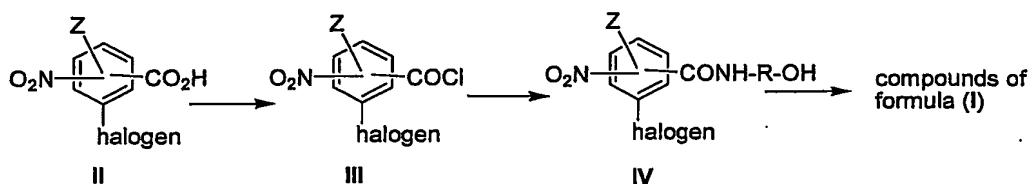
The expression "substantially minimal bystander effect" is to be understood as meaning that the killing of adjoining non-targeted cells is minimal because there is little or no diffusion between the targeted and non-targeted cells of an activated metabolite that arises from the enzymatic activation of a compound of Formula (I) or Formula (II) as defined above or a compound of any one of compounds Ia-Ic, or IIa-IIc as defined above or a mixture thereof.

Pharmaceutically acceptable salts of formula (I) include the basic or acidic compounds of formula (I) that form pharmaceutically acceptable salts with both organic and inorganic acids and/or organic and inorganic bases. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic, and the like. Examples of suitable bases for salt formation are sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine, and the like.

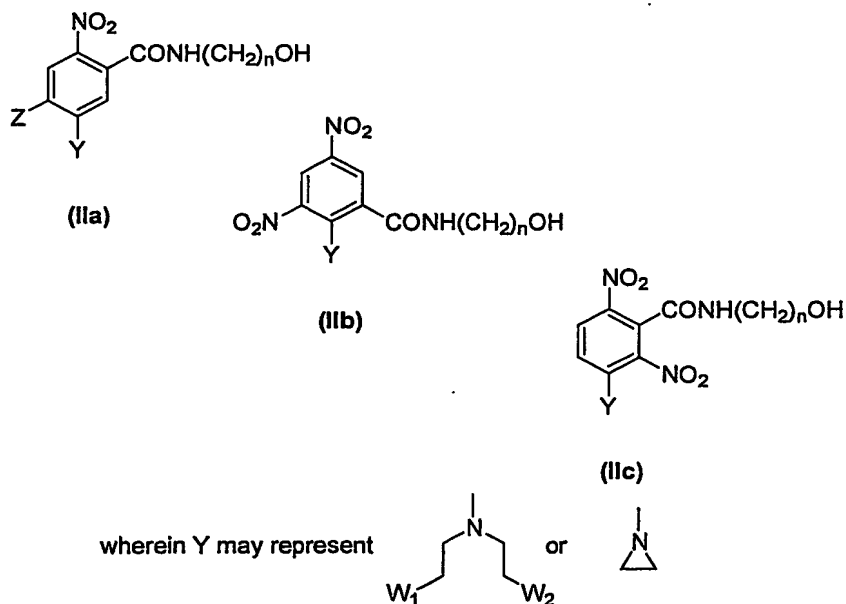
Further aspects of the present invention will become apparent from the following description given by way of example only and with reference to the accompanying synthetic schemes.

- 5 Examples of the compounds of Formula (I) where X is $-\text{CONH}-$ can be prepared by the processes described in Scheme 1, where Z is as defined above for Formula (I).

Scheme 1



- The following Tables 1a and 2a set out physical data for compounds within the general
- 10 Formula (I) and (II), representative of it, and capable of being prepared by the processes of the invention.



- 15 Table 1a. Representative examples of parent alcohols

No	Z	Y	Y (W ₁ ,W ₂)	n	mp	formula or ref	analyses
IIa-1	NO ₂	aziridine s	-	2	192-193	Ref. 1	C, H, N
IIa-2	NO ₂	-	Cl,Cl	2		Ref. 2	
IIa-3	NO ₂	-	Cl,Cl	3	90-91	Ref 4	C, H, N, Cl
IIa-7	NO ₂	-	Br,Br	2	151-152	C ₁₃ H ₁₆ Br ₂ N ₄ O ₆	C, H, N, Br
IIa-7s	SO ₂ Me	-	Br,Br	2	126-127	C ₁₄ H ₁₉ Br ₂ N ₃ O ₆ S	C, H, N
IIa -8	NO ₂	-	Br,Br	3	85-86	Ref 4	C, H, N, Br
IIa-9	NO ₂	-	Br,Br	4	123-124	C ₁₅ H ₂₀ Br ₂ N ₄ O ₆	C, H, N, Br
IIa-10	NO ₂	-	Br,Br	5	gum	C ₁₆ H ₂₂ Br ₂ N ₄ O ₆	HRMS
IIa-11	NO ₂	-	Br,Br	6	gum	C ₁₇ H ₂₄ Br ₂ N ₄ O ₆	HRMS
IIa-12	NO ₂	-	Br,OMs	2		Ref. 2	
IIa-13	NO ₂	-	Br,OMs	3	gum	C ₁₆ H ₂₁ BrN ₄ O ₉ S	HRMS
IIa 14	NO ₂	-	I,I	2	142-143	C ₁₃ H ₁₆ I ₂ N ₄ O ₆	C, H, N, I
IIb -1	-	aziridine s	-	6	189-192	C ₁₅ H ₂₀ N ₄ O ₆	C, H, N
IIb -2	-	-	Cl,Cl	2	109-111	C ₁₃ H ₁₆ Cl ₂ N ₄ O ₆	C, H, N
IIb-3	-	-	Cl,Cl	3	89-91	C ₁₄ H ₁₈ Cl ₂ N ₄ O ₆	C, H, N, Cl
IIb-4	-	-	Cl,Cl	4	gum	C ₁₅ H ₂₀ Cl ₂ N ₄ O ₆	HRMS
IIb-5	-	-	Cl,Cl	5	gum	C ₁₆ H ₂₂ Cl ₂ N ₄ O ₆	HRMS
IIb-6	-	-	Cl,Cl	6	gum	C ₁₇ H ₂₄ Cl ₂ N ₄ O ₆	HRMS
IIb-2m			Cl,OMs	2	gum	C ₁₄ H ₁₉ ClN ₄ O ₉ S	HRMS
IIb-7	-	-	Br,Br	2	105-108	C ₁₃ H ₁₆ Br ₂ N ₄ O ₆	C, H, N, Br
IIb-7a	-	-	Br,Br ^A	2	127-130	C ₁₅ H ₂₀ Br ₂ N ₄ O ₆	C, H, N
IIb -8	-	-	Br,Br	3	89-94	C ₁₄ H ₁₈ Br ₂ N ₄ O ₆	C, H, N, Br
IIb -9	-	-	Br,Br	4	gum	C ₁₅ H ₂₀ Br ₂ N ₄ O ₆	HRMS
IIb-10	-	-	Br,Br	5	gum	C ₁₆ H ₂₂ Br ₂ N ₄ O ₆	HRMS
IIb-11	-	-	Br,Br	6	gum	C ₁₇ H ₂₄ Br ₂ N ₄ O ₆	HRMS
IIb-12	-	-	Br,OMs	2		Ref. 3	
IIb-13	-	-	Br,OMs	3	gum	C ₁₅ H ₂₁ BrN ₄ O ₉ S	HRMS

IIb-14	-	-	I,I	2	129-131	C ₁₃ H ₁₆ I ₂ N ₄ O ₆	C, H, N
IIb-15	-	-	I,OMs	2	gum	C ₁₄ H ₁₉ IN ₄ O ₉ S	HRMS
IIc-6			Cl,OMs	3	104-109	C ₁₅ H ₂₁ ClN ₄ O ₉ S	C, H, N
IIc-7	-	-	Br,Br	2	gum	C ₁₃ H ₁₆ Br ₂ N ₄ O ₆	HRMS
IIc-8	-	-	Br,Br	3	gum	C ₁₄ H ₁₈ Br ₂ N ₄ O ₆	HRMS
IIc-9	-	-	Br,Br	4	gum	C ₁₅ H ₂₀ Br ₂ N ₄ O ₆	HRMS
IIc-12	-	-	Br,OMs	2	94-97	C ₁₄ H ₁₉ BrN ₄ O ₉ S	C, H, N
IIc-13	-	-	Br,OMs	3	115-117	Ref. 3	C, H, N
IIc-14	-	-	Br,OMs	4	114-117	C ₁₆ H ₂₃ BrN ₄ O ₉ S	C, H, N
IIc-15			I,OMs	3	100-103	C ₁₅ H ₂₁ IN ₄ O ₉ S	C, H, N

^Aα-methyl mustard

Notes

References for known compounds.

- 5 1. Khan AH, Ross WCJ. Tumor-growth inhibitory nitrophenylaziridines and related compounds. Structure-activity relations. II. Chem.-Biol. Int., 1971, 4, 11-22.
- 2 NZ Patent No.240785
3. Co-pending NZ Application No. 521851
4. Wilson WR, Pullen SM, Hogg A, Helsby NA, Hicks KO, Denny WA. Quantitation of bystander effects in nitroreductase suicide gene therapy using three-dimensional cell cultures. Cancer Res., 2002, 62, 1425-1432.

The compounds of Table 1a can be prepared by the general methods set out in Schemes 2a-2k, and exemplified in Examples 1-20 below.

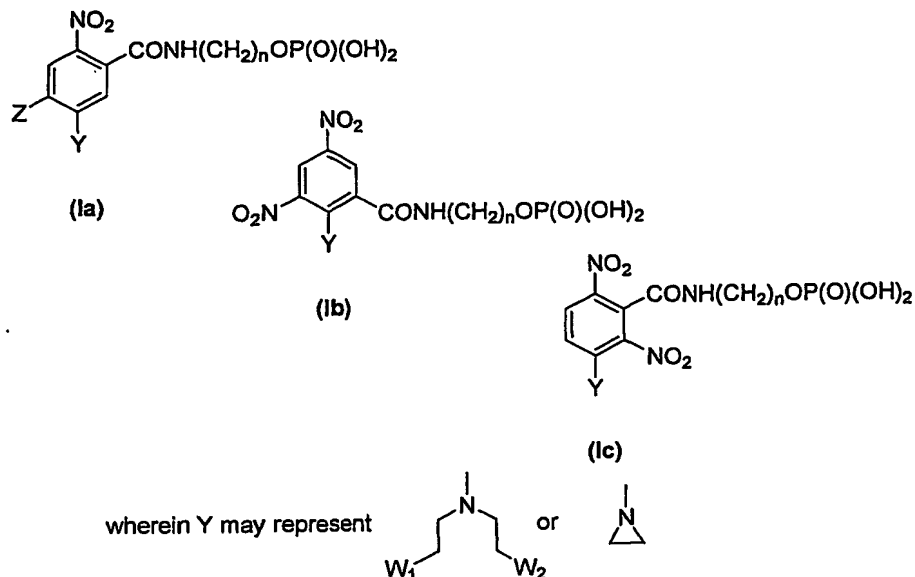


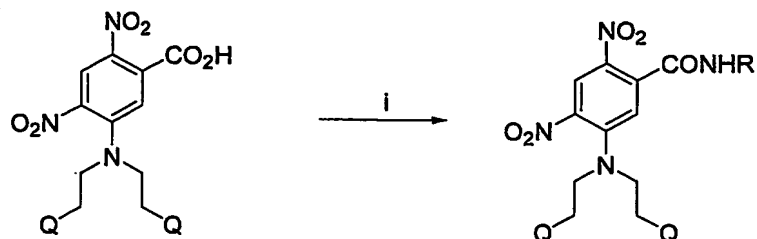
Table 1b. Examples of phosphates of formulae Ia-Ic

No	Z	Y (W ₁ , W ₂)	n	mp	formula	analyses
Ia-3P	NO ₂	Cl, Cl	3	195-200	C ₁₄ H ₁₉ Cl ₂ N ₄ O ₉ P	HRMS
Ia-8P	NO ₂	Br, Br	3	170-174	C ₁₄ H ₁₉ Br ₂ N ₄ O ₉ P	HRMS
Ib-2P	-	Cl, Cl	2	Foam	C ₁₃ H ₁₇ Cl ₂ N ₄ O ₉ P	HRMS
Ib-2mP	-	Cl, OMs	2	132-134	C ₁₄ H ₂₀ ClN ₄ O ₁₂ PS	C, H, N
Ib-7P	-	Br, Br	2	Foam	C ₁₃ H ₁₇ Br ₂ N ₄ O ₉ P	HRMS
Ib-7aP	-	Br, Br ^A	2	157-161	C ₁₅ H ₂₁ Br ₂ N ₄ O ₉ P	C, H, N
Ib-12P	-	Br, OMs	2	Foam	C ₁₄ H ₂₀ BrN ₄ O ₁₂ PS	HRMS
Ib-14P	-	I, I	2	Foam	C ₁₃ H ₁₇ I ₂ N ₄ O ₉ P	HRMS
Ib-15P	-	I, OMs	2	147-150	C ₁₄ H ₂₀ IN ₄ O ₁₂ PS	C, H, N
Ic-6P	-	Cl, OMs	3	88-92	C ₁₅ H ₂₂ ClN ₄ O ₁₂ PS	C, H, N
Ic-8P	-	Br, Br	3	Foam	C ₁₄ H ₁₉ Br ₂ N ₄ O ₉ P	HRMS
Ic-12P	-	Br, OMs	2	93-97	C ₁₄ H ₂₀ BrN ₄ O ₁₂ PS	C, H, N
Ic-13P	-	Br, OMs	3	foam	C ₁₅ H ₂₂ BrN ₄ O ₁₂ PS	HRMS
Ic-15P	-	I, OMs	3		C ₁₅ H ₂₂ IN ₄ O ₁₂ PS	

^Aalpha-Me

The compounds of Table 1b can be prepared by the general methods set out in Scheme 3, and exemplified in Examples 26-39 below.

Scheme 2a



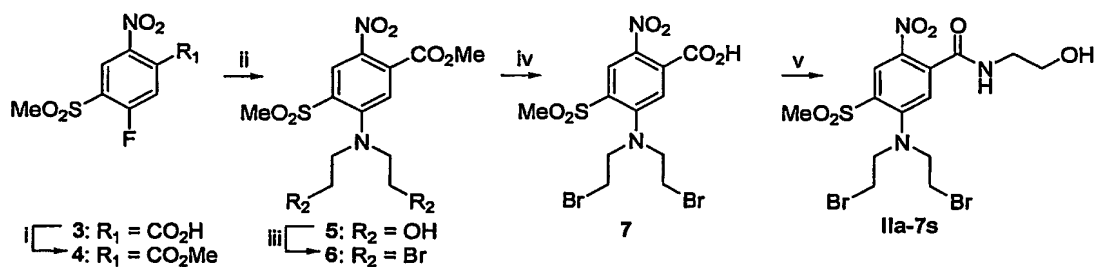
1: Q = Cl
2: Q = Br

IIa-2: R = (CH₂)₂OH, Q = Cl
IIa-3: R = (CH₂)₃OH, Q = Cl
IIa-7: R = (CH₂)₂OH, Q = Br
IIa-8: R = (CH₂)₃OH, Q = Br
IIa-9: R = (CH₂)₄OH, Q = Br
IIa-10: R = (CH₂)₅OH, Q = Br
IIa-11: R = (CH₂)₆OH, Q = Br

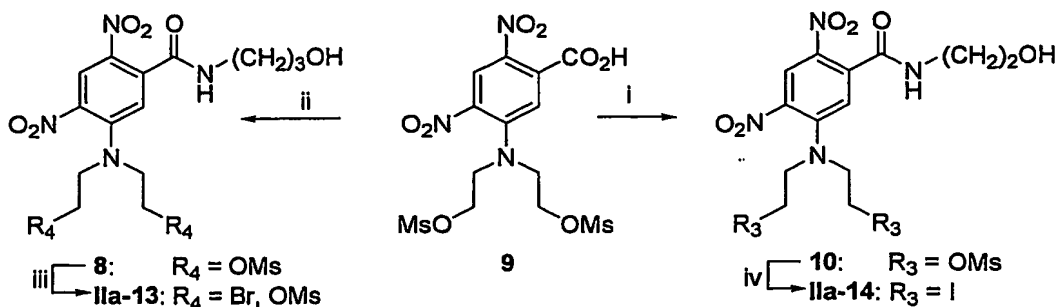
(i) SOCl₂ or oxalyl bromide, then RNH₂.

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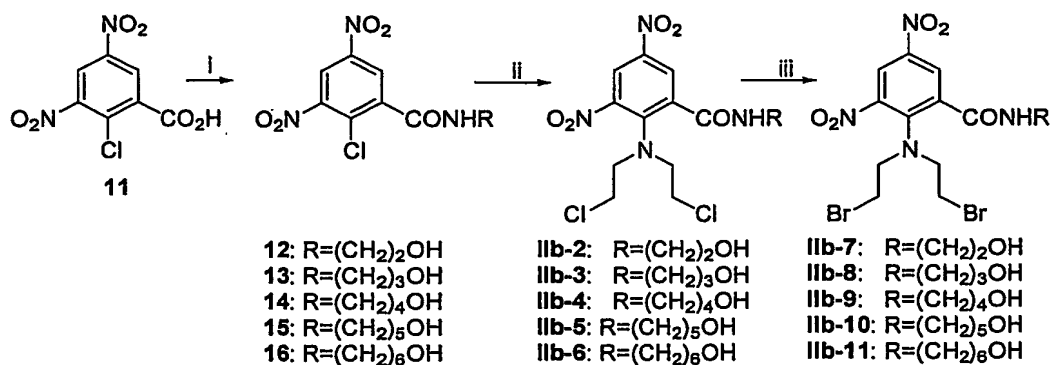
Scheme 2b



(i) SOCl₂/MeOH;
(ii) diethanolamine;
(iii) MsCl/py, then LiBr;
(iv) KOH;
(v) (COCl)₂/cat. DMF, then 2-aminoethanol

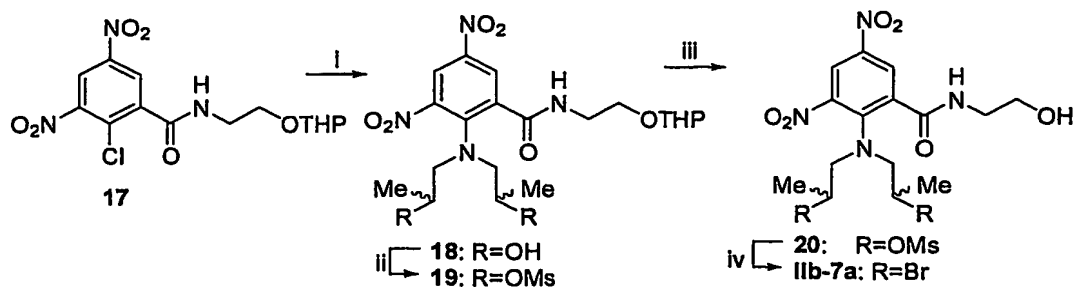
Scheme 2c

- (i) $\text{SOCl}_2/\text{cat DMF}$ or $(\text{COCl})_2/\text{cat DMF}$, then 2-aminoethanol
(ii) $\text{SOCl}_2/\text{cat DMF}$ or $(\text{COBr})_2/\text{cat DMF}$, then 3-amino-1-propanol
(iii) LiBr (1.4 equiv.)/DMF;
(iv) NaI (excess)/MeCN.

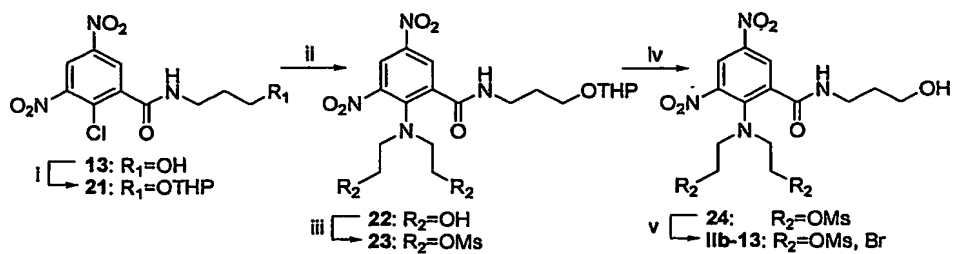
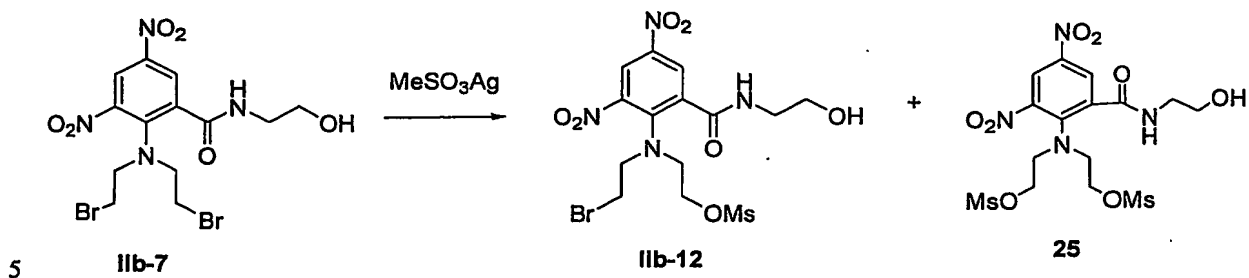
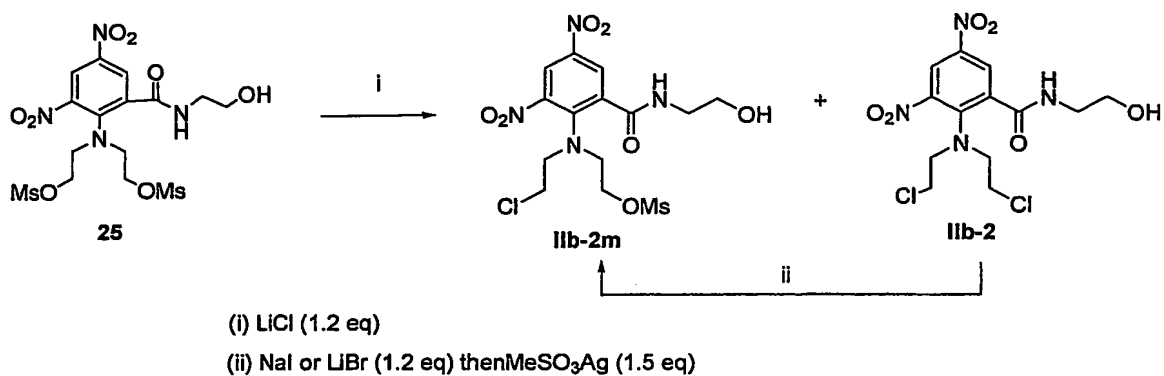
Scheme 2d

- (i) $\text{SOCl}_2/\text{cat. DMF}$, then RNH_2 ;
(ii) $\text{HN}(\text{CH}_2\text{CH}_2\text{Cl})_2$;
(iii) $\text{LiBr}/3\text{-Mebutanone}$

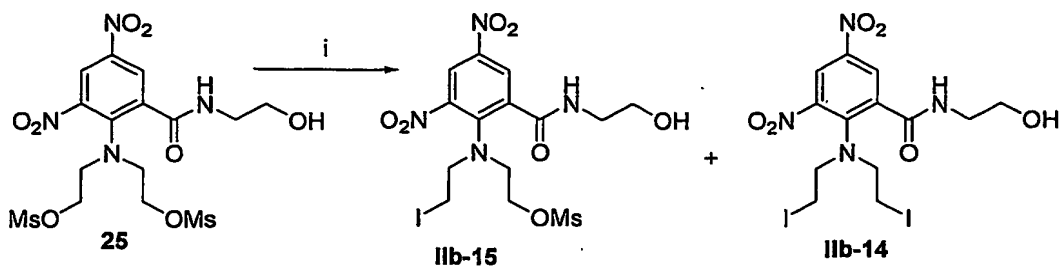
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Scheme 2e

- (i) diisopropanolamine;
(ii) $\text{MsCl}/\text{Et}_3\text{N}$;
(iii) $1\text{N HCl}/\text{THF}$;
(iv) LiBr (excess).

Scheme 2f**Scheme 2g****Scheme 2h**

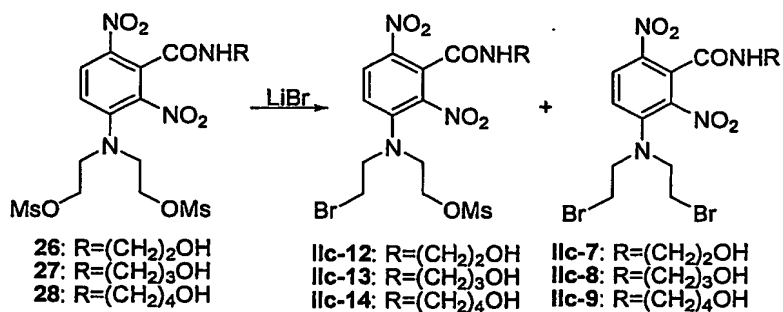
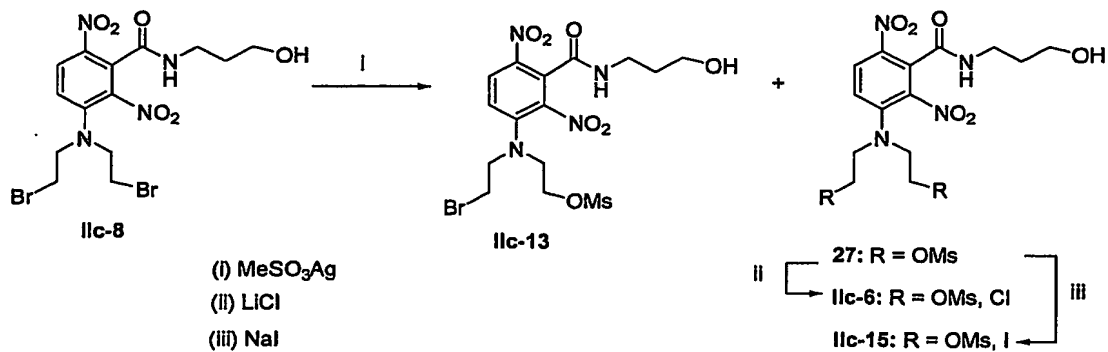
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Scheme 2i

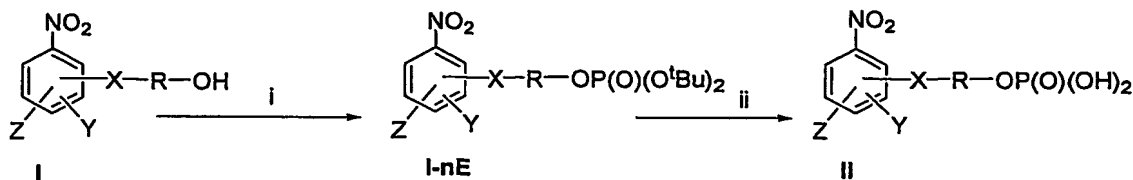
(i) NaI (1.5 eq)

Scheme 2j

5

**Scheme 2k**

10

Scheme 3

(i) $\text{Et}_2\text{NP}(\text{O}^t\text{Bu})_2$ or $t\text{-Pr}_2\text{NP}(\text{O}^t\text{Bu})_2/1\text{H-tetrazole}$, then mCPBA or H_2O_2

(ii) $\text{TFA}/\text{CH}_2\text{Cl}_2$

5 In Scheme 3, X, Y, Z, and R are as specified for formula (I) and (II) above.

Examples

The invention and the best mode for practising the same are illustrated by the following

10 Examples 1-25 (alcohols) and Examples 26-39 (phosphates).

Example 1 (Scheme 2a). N-(3-Hydroxypropyl)-5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (IIa-3). A suspension of 5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzoic acid [Palmer et al., J. Med. Chem., 1994, 37, 2175] (1) (2.50 g, 7.1 mmol) in SOCl_2 (20 mL) containing DMF (2 drops) was heated under reflux for 1 h, then concentrated to dryness under reduced pressure and re-evaporated with benzene. The resulting crude benzoyl chloride was dissolved in Me_2CO (50 mL) and the cooled (-5°C) solution was treated with a cold solution of 3-amino-1-propanol (1.09 g, 14.5 mmol) in water (25 mL). The reaction mixture was shaken at room temperature for 5 min, then diluted with water (25 mL), concentrated to half volume, and extracted with CH_2Cl_2 (2x). The organic extract was washed with 0.1 N HCl and water then worked up to give a solid which was chromatographed on silica gel, eluting with EtOAc to give IIa-3 (2.37 g, 82%): mp (EtOAc/ i -Pr $_2$ O) $90-91^\circ\text{C}$; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.63 (t, $J = 5.6$ Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.42 (s, 1 H, H-6), 4.46 (t, $J = 5.1$ Hz, 1 H, OH), 3.82 (t, $J = 5.9$ Hz, 4 H, $\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$), 3.68 (t, $J = 5.9$ Hz, 4 H, $\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$), 3.49 (q, $J = 6.0$ Hz, 2 H, CH_2OH), 3.29 (q, partially obscured, $J = 5.9$ Hz, 2 H, CONHCH_2), 1.68 (pent, $J = 6.7$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2$). Anal. ($\text{C}_{14}\text{H}_{18}\text{Cl}_2\text{N}_4\text{O}_6$) C, H, N, Cl.

Example 2 (Scheme 2a). N-(3-Hydroxypropyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-8). A suspension of powdered 5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzoic acid (**2**) (1.10 g, 2.49 mmol) in benzene (170 mL) was treated at 20 °C with oxalyl bromide (1.10 mL, 11.7 mmol) and DMF (2 drops). The mixture was stirred at 20 °C for 2 h, then concentrated under reduced pressure, and re-evaporated to dryness in the presence of benzene under high vacuum. The resulting acid bromide was dissolved in Me₂CO (20 mL) and the solution was treated at -5 °C with a cold solution of 3-amino-1-propanol (0.39 g, 5.19 mmol) in water (10 mL). The mixture was shaken at room temperature for 5 min, then diluted with water and extracted with EtOAc (2x). The organic extract was worked up and the resulting residue was chromatographed on silica gel, eluting with EtOAc, to give **IIa-8** (1.06 g, 85%): mp (EtOAc/i-Pr₂O) 85-86 °C; ¹H NMR [(CD₃)₂SO] δ 8.64 (t, *J* = 5.6 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.41 (s, 1 H, H-6), 3.77-3.64 (m, 8 H, N(CH₂CH₂Cl)₂), 4.46 (br s, 1 H, OH), 3.49 (t, *J* = 6.3 Hz, 2 H, CH₂OH), 3.33-3.25 (m, partially obscured, 2 H, CONHCH₂), 1.68 (pent, *J* = 6.72 Hz, 2 H, CH₂CH₂CH₂). Anal. (C₁₄H₁₈Br₂N₄O₆) C, H, N, Br.

Example 3 (Scheme 2a). N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-7). Similar reaction of the acid bromide of **2** with 2-aminoethanol gave **IIa-7** (0.78 g, 46%): mp (MeOH/EtOAc/pet. ether) 151-152 °C; ¹H NMR [(CD₃)₂SO] δ 8.73 (t, *J* = 5.7 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.76 (t, *J* = 5.6 Hz, 1 H, OH), 3.77-3.64 (m, 8 H, N(CH₂CH₂Br)₂), 3.53 (q, *J* = 6.0 Hz, 2 H, CH₂OH), 3.31 (q, partially obscured, *J* = 6.1 Hz, 2 H, CONHCH₂). Anal. (C₁₃H₁₆Br₂N₄O₆) C, H, N, Br.

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Example 4 (Scheme 2a). N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-9). Similar reaction of the acid bromide of **2** with 4-amino-1-butanol in cold Me₂CO, followed by chromatography on silica gel and elution with EtOAc gave **IIa-9** (69%) as a yellow solid: mp (EtOAc/iPr₂O) 123-124 °C; ¹H NMR [(CD₃)₂SO] δ 8.62 (t, *J* = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.39 (s, 1 H), 4.39 (t, *J* = 5.1 Hz, 1 H), 3.78-3.64 (m, 8 H), 3.47-3.40 (m, 2 H), 3.27-3.20 (m, 2 H), 1.61-1.44 (m, 4 H). Anal. (C₁₅H₂₀Br₂N₄O₆) C, H, N, Br.

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- Example 5 (Scheme 2a).** N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (**IIa -10**). Similar reaction of the acid bromide of **2** with 5-amino-1-pentanol in cold Me₂CO, followed by chromatography on silica gel and elution with EtOAc gave **IIa -10** (66%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.62 (t, *J* = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.34 (t, *J* = 5.1 Hz, 1 H), 3.79-3.64 (m, 8 H), 3.44-3.37 (m, 2 H), 3.26-3.18 (m, 2 H), 1.59-1.29 (m, 4 H). HRMS (FAB) Calcd. for C₁₆H₂₃⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 524.9984, found 524.9964.
- Example 6 (Scheme 2a).** N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (**IIa -11**). Similar reaction of the acid bromide of **2** with 6-amino-1-hexanol in cold Me₂CO, followed by chromatography on silica gel and elution with EtOAc gave **IIa -11** (72%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.61 (t, *J* = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.31 (t, *J* = 5.2 Hz, 1 H), 3.79-3.64 (m, 8 H), 3.43-3.36 (m, 2 H), 3.27-3.19 (m, 2 H), 1.58-1.26 (m, 4 H). HRMS (FAB) Calcd. for C₁₇H₂₅⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 539.0141, found 539.0137.

- Example 7. (Scheme 2b).** 5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzamide (**IIa -7s**). 5-Fluoro-4-(methylsulfonyl)-2-nitrobenzoic acid [Atwell et al., ACDD, 1996, 11, 553] (**3**) was heated in excess SOCl₂/catalytic DMF to provide the acid chloride, which was reacted with dry MeOH to give methyl 5-fluoro-4-(methylsulfonyl)-2-nitrobenzoate (**4**): mp (EtOAc/hexane) 134-135 °C; ¹H NMR [(CD₃)₂SO] δ 8.49 (d, *J* = 5.9 Hz, 1 H), 8.14 (d, *J* = 9.3 Hz, 1 H), 3.92 (s, 3 H), 3.46 (s, 3 H). Anal. (C₉H₈FNO₆S) C, H, N.

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- A mixture of **4** (1.48 g, 5.34 mmol) and diethanolamine (1.40 g, 13.3 mmol) in DMA (6 mL) was stirred at 30 °C for 1 h, and then diluted with EtOAc (60 mL). The solution was washed with brine (2x) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH, followed by recrystallization from EtOAc/iPr₂O, to give methyl 5-[bis(2-hydroxyethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoate (**5**) (1.41 g, 73%): mp 99-100 °C; ¹H NMR [(CD₃)₂SO] δ 8.56 (s, 1 H), 7.73

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(s, 1 H), 4.62 (t, $J = 4.9$ Hz, 2 H), 3.89 (s, 3 H), 3.59-3.49 (m, 8 H), 3.45 (s, 3 H). Anal. ($C_{13}H_{18}N_2O_8S$) C, H, N.

A solution of 5 (1.48 g, 4.08 mmol) in dry pyridine (15 mL) was treated dropwise at 0 °C with MsCl (0.80 mL, 10.3 mmol). The reaction was stirred at 0 °C for 2 h, then poured into 10% aqueous NaBr. The resulting crude dimesylate was collected, washed well with water, dried, dissolved in DMF (15 mL) and stirred with NaBr (21.6 g, 25 mmol) at 70 °C for 1.5 h. The cooled mixture was poured into water and the resulting solid was purified by chromatography, on silica gel, eluting with CH_2Cl_2 , then recrystallisation from CH_2Cl_2/iPr_2O to give methyl 5-[bis(2-bromoethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoate (6) (1.47 g, 74%): mp 161-162 °C; 1H NMR [$(CD_3)_2SO$] δ 8.58 (s, 1 H), 7.94 (s, 1 H), 3.90 (s, 3 H), 3.82 (t, $J = 7.0$ Hz, 4 H), 3.63 (t, $J = 6.9$ Hz, 4 H), 3.48 (s, 3 H). Anal. ($C_{13}H_{16}Br_2N_2O_6S$) C, H, N.

A solution of 6 (1.00 g, 2.05 mmol) in a dioxane/MeOH (1:1, 20 mL) was treated at 10 °C with 4N aqueous KOH (5 mL), and stirred at 10 °C for 45 min. The mixture was acidified to pH 2 with 1 N aqueous HBr, concentrated to a small volume under reduced pressure, and then diluted with saturated aqueous NaBr (20 mL). The resulting semi-solid was isolated and crystallized twice from MeOH/ H_2O to give 5-[bis(2-bromoethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoic acid (7) (0.70 g, 72%): mp 174-176 °C; 1H NMR [$(CD_3)_2SO$] δ 8.50 (s, 1 H), 7.88 (s, 1 H), 3.79 (t, $J = 7.0$ Hz, 4 H), 3.62 (t, $J = 7.0$ Hz, 4 H), 3.48 (s, 3 H). Anal. ($C_{12}H_{14}Br_2N_2O_6S$) C, H, N.

A finely-divided suspension of 7 (260 mmg, 0.55 mmol) in dry benzene (50 mL) was treated with $(COBr)_2$ (2.13 mL, 0.20 mmol) and catalytic DMF. The mixture was stirred for 2 h, then concentrated to dryness under reduced pressure and re-evaporated with benzene under high vacuum. The resulting crude acid bromide was dissolved in Me_2CO (10 mL) and treated at -5 °C with a cold solution of 2-aminoethanol (101 mg, 1.65 mmol) in water (5 mL). The mixture was stirred at 0 °C for 5 min, then acidified to pH 4 with 1 N aqueous HBr, and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc, to give IIa -7s (222 mg, 78%): mp (EtOAc/ iPr_2O) 126-127 °C; 1H NMR [$(CD_3)_2SO$] δ 8.75 (t, $J = 5.6$ Hz, 1 H), 8.51 (s, 1 h),

7.68 (s, 1 H), 4.79 (t, $J = 5.4$ Hz, 1 H), 3.76 (t, $J = 7.1$ Hz, 4 H), 3.62 (t, $J = 7.0$ Hz, 4 H), 3.54 (q, $J = 5.9$ Hz, 2 H), 3.48 (s, 3 H), 3.31 (after D₂O exchange, t, $J = 6.0$ Hz, 2 H).

HRMS (FAB) calcd. for C₁₄H₂₀⁷⁹Br₂N₃O₆S (MH⁺) m/z 515.9440; found 515.9425.

- 5 **Example 8 (Scheme 2c).** 2[(2-Bromoethyl)-5-[[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate (IIa-13) and 5-[bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2, 4-dinitrobenzamide (IIa-14). 5-(Bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoic acid [A method of preparing this compound is disclosed in co-pending NZ Application No. 521851] (9) was heated under
10 reflux in excess SOCl₂ (60 mL) and catalytic DMF for 1 h. Evaporation under reduced pressure, followed by azeotroping in benzene, gave the crude acid chloride. This was dissolved in dry Me₂CO and treated at 0 °C with 3-amino-1-propanol at 0 °C for 5 min. The mixture was acidified to pH 2-3 with 0.2 N HCl, concentrated to half volume, and then solid NaBr was added, followed by extraction with EtOAc (2x). Evaporation, and
15 chromatography of the residue on silica gel, eluting with EtOAc/MeOH (9:1), gave give 2-(5-{[(3-hydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (8) (68%) as a yellow gum; ¹H NMR [(CD₃)₂SO] δ 8.54 (t, $J = 5.7$ Hz, 1 H), 8.53 (s, 1 H), 7.45 (s, 1 H), 4.43 (t, $J = 5.1$ Hz, 1 H), 4.33 (t, $J = 5.2$ Hz, 4 H), 3.69 (t, $J = 5.2$ Hz, 4 H), 3.57 (q, $J = 5.9$ Hz, 2 H), 3.26 (after D₂O
20 exchange, t, $J = 7.0$ Hz, 2 H), 3.12 (s, 6 H), 1.66 (pent, $J = 6.7$ Hz, 2 H). HRMS (FAB) calcd. for C₁₆H₂₅N₄O₁₂S (MH⁺) m/z 529.0910; found 529.0904.

A solution of 8 in DMF was treated with LiBr (1.4 equiv.), and worked up as above, and the product was chromatographed on silica gel. Elution with EtOAc gave a small amount
25 of the dibromo mustard, while elution with EtOAc/MeOH (19:1) gave IIa-13 (31%) as a yellow gum: ¹H NMR [(CD₃)₂SO] δ 8.60 (t, $J = 5.6$ Hz, 1 H), 8.54 (s, 1 H), 7.44 (s, 1 H), 4.45 (t, $J = 5.2$ Hz, 1 H), 4.33 (t, $J = 5.1$ Hz, 2 H), 3.74 (t, $J = 5.2$ Hz, 2 H), 3.72-3.66 (m, 4 H), 3.49 (q, $J = 5.9$ Hz, 2 H), 3.27 (after D₂O exchange, t, $J = 7.0$ Hz, 2 H), 3.14 (s, 3 H), 1.68 (pent, $J = 6.7$ Hz, 2 H). HRMS (FAB) calcd. for C₁₅H₂₂⁷⁹BrN₄O₉S (MH⁺) m/z
30 515.0270; found 515.0283.

Similar treatment of the acid chloride of 9 (activation with $(\text{COCl})_2/\text{DMF}$) with 2-aminoethanol gave 2-(5-{[(2-hydroxyethyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (10). A stirred mixture of 10 (1.42 g, 2.76 mmol) and NaI (3.3g, 22 mmol) in dry MeCN (45 mL) was heated at reflux for 1h, then concentrated under reduced pressure. The residue was partitioned between EtOAc and water, and the organic layer was washed with water and evaporated. The residue was chromatographed on silic gel, eluting with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (1:4), followed by recrystallisation from MeOH/EtOAc/*i*-Pr₂O to give **IIa -14** (2.9 g, 81%): mp 142-143 °C; ¹H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.73 (t, $J = 5.7$ Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.76 (t, $J = 5.5$ Hz, 1 H), 3.68 (t, $J = 6.9$ Hz, 4 H), 3.57-3.49 (m, 2 H), 3.39 (t, $J = 6.9$ Hz, 4 H), 3.34-3.26 (m, partially obscured, 2 H). Anal. ($\text{C}_{13}\text{H}_{16}\text{I}_2\text{N}_4\text{O}_6$) C, H, N.

Example 9. 2-(Aziridin-1-yl)-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide (IIa -1). A solution of 2-chloro-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide (16) [for preparation see Example 14 below] (118 mg, 0.34 mmol) and Et₃N (200 mg) in EtOAc (200 mL) was treated with aziridine (100 mg) at room temperature for 3 h. The mixture was diluted with EtOAc and washed three times with water, after dry, concentrated under reduced pressure until about 20 mL, the yellow solid was collected and gave 101 mg product (84%); ¹H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.74 (d, $J = 2.8$ Hz, 1 H), 8.63 (m, 1 H), 8.29 (d, $J = 2.8$ Hz, 1 H), 4.31 (m, 1 H), 3.39 (m, 2 H), 3.25 (m, 2 H), 2.37 (s, 4 H), 1.56 (m, 2 H), 1.43 (m, 2 H), 1.33 (m, 4 H). Anal. ($\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_6$) C, H, N.

Example 10 (Scheme 2d). 2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-2) and 2-[bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-7). 2-Chloro-3,5-dinitrobenzoic acid (1) (18 g, 81 mmol) was treated with SOCl_2 (250 ml) containing one drop of DMF and heated under reflux for 6 h. Evaporation of reagent followed by azeotrope with benzene gave the crude acid chloride, which was dissolved in THF (200 mL) and added dropwise to a solution made of 25 mL of 2-aminoethanol in THF (400 mL) and cooled with dryice-acetone bath. After stirring for 20 min. the reaction mixture was acidified to pH 4-5 with 1 N HCl, most of the solvent was evaporated, and the residue was partitioned between water (250 mL) and EtOAc (300 mL). The aqueous phase was extracted with EtOAc, and the combined

organic phases were washed with sat. NaHCO_3 , 1 N HCl and brine respectively, then concentrated to give 2-chloro-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (**2**) 21.34 g (91%) as a white solid: mp (EtOAc) 159-160 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.99 (d, $J = 2.6$ Hz, 1 H, H-4), 8.86 (m, 1 H, CONH), 8.56 (d, $J = 2.6$ Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.54
5 (m, 4 H). Anal. ($\text{C}_9\text{H}_8\text{ClN}_3\text{O}_6$) C, H, N.

A solution of **12** (1.52 g, 5.3 mmol) and Et_3N (4 mL) in *p*-dioxane (60 mL) was treated with *N,N*-bis(2-chloroethyl)amine hydrochloride (3.0 g, 16.5 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to give the crude product,
10 which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) and concentration of the eluate under reduced pressure gave a oily residue that was dissolved in minimum amount of EtOAc. Petroleum ether was added slowly until incipient cloudiness, and the solution was stood overnight to precipitate 2-[bis(2-chloroethyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (**IIb-2**) (2.07 g, 100%) as
15 yellow crystals: mp (EtOAc/petroleum ether) 109-111 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (d, $J = 2.6$ Hz, 1 H, H-4), 8.72 (m, 1 H, CONH), 8.34 (d, $J = 2.6$ Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.72 (m, 4 H, $2 \times \text{CH}_2\text{Cl}$), 3.55 (m, 2 H), 3.42 (m, 4 H, $2 \times \text{CH}_2\text{N}$), 3.34 (m, 2 H); ^{13}C NMR δ 165.3, 145.8, 145.3, 141.0, 136.3, 127.5, 122.1, 59.1, 54.1, 42.1, 41.5. HRMS (FAB) $[\text{MH}^+]$ Calcd. For $\text{C}_{13}\text{H}_{17}^{35}\text{Cl}_2\text{N}_4\text{O}_6$ m/z 395.0525. Found; 395.0525.

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A solution of **IIb-2** (1.20 g, 3.0 mmol) and LiBr (5.0 g, 58 mmol) in 3-methyl-2-butanone (20 mL) was heated under reflux for 6 h, then cooled and poured into water. Extraction with EtOAc gave a crude product (<95 % pure), that was re-treated with LiBr (5.0 g, 58 mmol) in 3-methyl-2-butanone for a further 4 h, then worked up and chromatographed on
25 silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 1:0), to give **IIb-7** (1.39 g, 95%): mp (EtOAc/petroleum ether) 105-108 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.73 (m, 1 H, CONH), 8.34 (d, $J = 2.7$ Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.59-3.29 (m, 12 H); ^{13}C NMR δ 165.3, 145.4, 145.3, 141.1, 136.5, 127.4, 122.1, 59.3, 53.9, 42.1, 30.0. HRMS (FAB) Calcd. For $\text{C}_{13}\text{H}_{17}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 482.9515.
30 Found; 482.9492. Anal. ($\text{C}_{13}\text{H}_{16}\text{Br}_2\text{N}_4\text{O}_6$) H, N, Br; C: found, 32.9; calculated 32.3%.

Example 11 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide (IIb-3) and 2-[bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide (IIb-8). Reaction of the acid chloride of 11 (17 g) with 3-aminopropanol (7.5 g) in Me₂CO (120 mL) at 0 °C as described above, gave 2-chloro-N-(3-hydroxypropyl)-3,5-dinitrobenzamide (13) (5.06 g, 26%): mp (EtOAc/petroleum ether) 120-121 °C; ¹H NMR [(CD₃)₂SO] δ 8.99 (d, *J* = 2.6 Hz, 1 H, H-4), 8.79 (m, 1 H, CONH), 8.51 (d, *J* = 2.6 Hz, 1 H, H-6), 4.50 (m, 1 H, OH), 3.49 (m, 2 H), 3.32 (m, 2 H), 1.70 (m, 2 H). Anal. (C₁₀H₁₀ClN₃O₆) C, H, N.

A solution of 13 (1.39 g, 4.58 mmol) and Et₃N (4 mL) in *p*-dioxane (60 mL) was treated with N,N-bis(2-chloroethyl)amine hydrochloride (2.9 g, 16.0 mmol) at 50 °C for 24 h. Workup as described above gave **IIb-3** (1.84 g, 100%): mp (EtOAc/petroleum ether) 89-91 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.71 (m, 1 H, CONH), 8.30 (d, *J* = 2.7 Hz, 1 H, H-6), 4.52 (m, 1 H, OH), 3.71 (m, 4 H, 2xCH₂Cl), 3.50 (m, 2 H), 3.42 (m, 4 H, 2xCH₂N), 3.32 (m, 2 H), 1.71 (m, 2 H); ¹³C NMR δ 165.1, 145.7, 145.5, 141.0, 136.4, 127.3, 122.1, 58.4, 54.1, 41.5, 36.7, 31.8. HRMS (FAB) Calcd. For C₁₄H₁₉³⁵Cl₂N₄O₆ [M+H⁺] *m/z* 409.0682. Found; 409.0678.

Treatment of **IIb-3** with LiBr in 3-methyl-2-butanone twice, as described above, gave **IIb-8** (74% yield): mp (EtOAc/petroleum ether) 89-94 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.72 (m, 1 H, CONH), 8.30 (d, *J* = 2.7 Hz, 1 H, H-6), 3.77-3.44 (m, 12 H), 1.70 (m, 2 H); ¹³C NMR δ 165.1, 145.5, 145.3, 141.2, 136.5, 127.3, 122.1, 58.4, 54.0, 36.7, 31.8, 29.9. HRMS (FAB) Calcd. For C₁₄H₁₉⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 496.9671. Found; 496.9658.

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Example 12 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide (IIb-4) and 2-[bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide (IIb-9). Reaction of the acid chloride of 11 (2.65 g, 10 mmol) with 4-aminobutanol (1.9 g) as above, followed by acidification to pH 4-5 with 1 N HCl and evaporation of most of the solvent gave a residue. This was partitioned between water (50 mL) and EtOAc (100 mL). The aqueous phase was extracted with EtOAc, and the combined organic phase were washed with sat. NaHCO₃, 1 N HCl and brine respectively,

30

then concentrated to give 2-chloro-*N*-(4-hydroxybutyl)-3,5-dinitrobenzamide (**14**) 1.11 g (35%): mp (EtOAc) 121-124 °C; ¹H NMR [(CD₃)₂SO] δ 8.98 (d, *J* = 2.7 Hz, 1H), 8.79 (m, 1 H), 8.52 (d, *J* = 2.7 Hz, 1H), 4.43 (m, 1 H), 3.43 (m, 2H), 3.26 (m, 2 H), 1.54 (m, 4 H); ¹³C NMR δ 162.6, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.2, 39.1, 29.8, 25.3. Anal.

5 (C₁₁H₁₂ClN₃O₆) C, H, N.

A solution of **14** (0.75 g, 2.3 mmol) and Et₃N (2 mL) in *p*-dioxane (30 mL) was treated with *N,N*-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc gave the crude product which

10 was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-4** (0.99 g, 100%) as yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.71 (d, *J* = 2.8 Hz, 1 H), 8.69 (m, 1 H), 8.27 (d, *J* = 2.8 Hz, 1 H), 4.37 (m, 1 H), 3.70 (m, 4 H), 3.38 (m, 6 H), 3.25 (m, 2 H), 1.56 (m, 2 H), 1.47 (m, 2 H); ¹³C NMR δ 165.0, 145.7, 145.5, 141.0, 136.4, 127.2, 122.0, 60.2, 54.2, 41.5, 39.2, 29.8, 25.2. HRMS (FAB) Calcd. For C₁₅H₂₁³⁵Cl₂N₄O₆ [M+H⁺] *m/z*

15 423.0838. Found; 423.0847.

A solution of **IIb-4** (0.96 g, 3.04 mmol) and LiBr (5 g) in 3-methyl-2-butanone (15 mL) was heated under reflux for 6 h, then cooled and poured into water. Extraction with EtOAc gave a crude product (<95 % pure), that was re-treated with LiBr (5 g) in 3-

20 methyl-2-butanone for a further 4 h, then worked up and chromatographed on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 3:1) give **IIb-9** (1.01 g, 87%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.8 Hz, 1 H), 8.72 (m, 1 H), 8.28 (d, *J* = 2.8 Hz, 1 H), 3.60-3.26 (m, 12 H), 1.58 (m, 2 H), 1.49 (m, 2 H); ¹³C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.2, 54.1, 39.2, 29.9, 29.8, 25.2. HRMS (FAB) Calcd. For

25 C₁₅H₂₁⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 510.9828. Found; 510.9832.

Example 13 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (**IIb-5**) and 2-[bis(2-bromoethyl)amino]-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (**IIb-10**). Similar reaction of the acid chloride of **11** with 5-

30 aminopentanol as above gave 2-chloro-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (**15**), 1.3 g (39%), mp (EtOAc) 105-108 °C; ¹H NMR [(CD₃)₂SO] δ 8.98 (d, *J* = 2.7 Hz, 1H), 8.79 (m, 1 H), 8.50 (d, *J* = 2.7 Hz, 1 H), 4.35 (m, 1 H), 3.39 (m, 2 H), 3.26 (m, 2 H), 1.54

(m, 2 H), 1.44 (m, 2 H), 1.36 (m, 2 H); ^{13}C NMR δ 162.7, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.5, 39.1, 32.0, 28.4, 22.8. Anal. ($\text{C}_{12}\text{H}_{14}\text{ClN}_3\text{O}_6$) C, H, N.

A solution of **15** (0.63 g, 2.3 mmol) and Et_3N (2 mL) in *p*-dioxane (30 mL) was treated
5 with *N,N*-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to give the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-5** (0.82 g, 100%) as yellow foam; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.73 (d, $J = 2.8$ Hz, 1 H), 8.69 (m, 1 H), 8.28 (d, $J = 2.8$ Hz, 1 H), 4.32 (m, 1 H), 3.70 (m, 4 H), 3.40 (m, 6 H), 3.25 (m, 2 H),
10 1.55 (m, 2 H), 1.47 (m, 2 H), 1.37 (m, 2 H); ^{13}C NMR δ 165.0, 145.7, 145.5, 141.0, 136.4, 127.2, 122.0, 60.5, 54.2, 41.5, 39.3, 32.0, 28.3, 22.9. HRMS (FAB) Calcd. For $\text{C}_{16}\text{H}_{23}^{35}\text{Cl}_2\text{N}_4\text{O}_6$ [$\text{M}+\text{H}^+$] m/z 437.0995. Found; 437.0991.

Similar reaction of **IIb-5** (1.3 g) with LiBr gave **IIb-10** (1.35 g, 86%) as a yellow foam;
15 ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.74 (d, $J = 2.8$ Hz, 1 H), 8.71 (m, 1 H), 8.28 (d, $J = 2.8$ Hz, 1 H), 3.60-3.26 (m, 12 H), 1.55 (m, 2 H), 1.48 (m, 2 H), 1.37 (m, 2 H); ^{13}C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.5, 54.1, 39.3, 32.0, 29.8, 28.4, 22.9. HRMS (FAB) Calcd. For $\text{C}_{16}\text{H}_{23}^{79}\text{Br}_2\text{N}_4\text{O}_6$ [$\text{M}+\text{H}^+$] m/z 524.9984. Found; 524.9975.

20 **Example 14 (Scheme 2d).** 2-[Bis(2-chloroethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**IIb-6**) and 2-[bis(2-bromoethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**IIb-11**). Similar reaction of the acid chloride of **11** with 6-aminohexanol as above gave 2-chloro-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**16**), 0.9 g (26%), mp (EtOAc) 88-91 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.98 (d, $J = 2.7$ Hz, 1H), 8.78 (m, 1 H), 8.49 (d, $J = 2.7$ Hz, 1 H), 4.32 (m, 1 H), 3.39 (m, 2H), 3.26 (m, 2 H), 1.54 (m, 2 H),
25 1.44 (m, 2 H), 1.34 (m, 4 H); ^{13}C NMR δ 162.7, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.5, 39.1, 32.3, 28.6, 26.2, 25.1. Anal. ($\text{C}_{13}\text{H}_{16}\text{ClN}_3\text{O}_6$) C, H, N.

A solution of **16** (0.67 g, 2.5 mmol) and Et_3N (2 mL) in *p*-dioxane (30 mL) was treated
30 with *N,N*-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to give the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-6**

(0.87 g, 100%) as yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (d, $J = 2.8$ Hz, 1 H), 8.70 (m, 1 H), 8.28 (d, $J = 2.8$ Hz, 1 H), 4.31 (m, 1 H), 3.70 (m, 4 H), 3.38 (m, 6 H), 3.25 (m, 2 H), 1.54 (m, 2 H), 1.40 (m, 2 H), 1.32 (m, 4 H); ^{13}C NMR δ 165.0, 145.7, 145.6, 141.0, 136.4, 127.2, 122.0, 60.5, 54.2, 41.5, 39.2, 32.3, 28.5, 26.3, 25.1. HRMS (FAB) Calcd. For $\text{C}_{17}\text{H}_{25}^{35}\text{Cl}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 451.1151. Found; 451.1154.

Similar reaction of **IIb-6** (0.97 g) with LiBr gave **IIb-11** (0.96 g, 81%) as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1 H), 8.70 (m, 1 H), 8.28 (d, $J = 2.8$ Hz, 1 H), 3.60-3.26 (m, 12 H), 1.54 (m, 2 H), 1.43 (m, 2 H), 1.32 (m, 4 H); ^{13}C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.6, 54.1, 39.2, 32.4, 29.9, 28.5, 26.3, 25.1. HRMS (FAB) Calcd. For $\text{C}_{17}\text{H}_{25}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 539.0141. Found; 539.0135.

Example 15 (Scheme 2e). 2-[Bis(2-bromopropyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-7a). Reaction of 2-chloro-3,5-dinitro-N-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]benzamide (**17**) (1.02 g) [For method of preparation see co-pending NZ Application No. 521851] with diisopropanolamine (0.8 g) as above gave 2-[bis(2-hydroxypropyl)amino]-3,5-dinitro-N-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]benzamide (**18**) (1.29 g, 100%): as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 9.22 (br, 1H), 8.66 (d, $J = 2.8$ Hz, 1H), 8.29 (d, $J = 2.8$ Hz, 1H), 4.99 (m, 1 H), 4.85 (br, 1 H), 4.62 (br, 1 H), 3.94 (m, 2 H), 3.77 (m, 2 H), 3.53 (m, 4H), 3.26 (m, 2 H), 1.48 (m, 10 H), 0.98 (m, 6 H); ^{13}C NMR δ 166.5, 147.8, 142.4, 138.2, 132.6, 128.8, 123.8, 98.1, 64.8, 63.5, 61.5, 60.1, 30.1, 25.0, 20.5, 20.2, 19.1. HRMS (FAB) Calcd. For $\text{C}_{20}\text{H}_{31}\text{N}_4\text{O}_9$ $[\text{M}+\text{H}^+]$ m/z 471.2091. Found; 471.2089.

Reaction of **18** with MsCl as above gave **1-methyl-2-[[2-[(methylsulfonyl)oxy]propyl]-2,4-dinitro-6-([2-(tetrahydro-2H-pyran-2-yloxy)ethyl]amino)carbonyl]anilino]ethyl methanesulfonate (19)** (2.52 g, 100%): as a yellow foam; which was used directly for the next step.

A solution of **19** (2.52 g, 4.03 mmol) in THF (150 mL) was treated with 1 N HCl (100 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. NaHCO_3 , and extracted with EtOAc (3x80 mL). The combined

organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(100:1), to give 2-(2-[[[(2-hydroxyethyl)amino]carbonyl]{2-[(methylsulfonyl)oxy]propyl}-4,6-dinitroanilino)-1-methylethyl methanesulfonate (**20**) (0.80 g, 37%): as a yellow foam;
5 ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.94 (m, 1 H), 8.72 (m, 1 H), 8.35 (m, 1 H), 4.92 (m, 2 H), 3.56 (m, 2 H), 3.30 (m, 6 H), 3.16 (s, 6 H), 1.32 (m, 6 H); ^{13}C NMR δ 165.9, 145.8, 143.4, 139.4, 133.6, 128.0, 123.1, 76.3, 59.2, 57.3, 42.2, 37.7, 18.6. HRMS (FAB) Calcd. For $\text{C}_{17}\text{H}_{27}\text{N}_4\text{O}_{12}\text{S}_2$ $[\text{M}+\text{H}^+]$ m/z 543.1067. Found; 543.1074.

10 Treatment of **20** (0.52 g, 0.96 mmol) with LiBr (0.5 g, 5.8 mmol) in EtOAc (50 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **IIB-7a** (0.31 g, 62%): as yellow solid: mp (EtOAc/petroleum ether) 127-130 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.91 (m, 1H, CONH), 8.70 (d, $J = 2.8$ Hz, 1H, H-4), 8.32 (d, $J = 2.8$ Hz, 1H, H-6), 4.80 (m, 1 H), 4.42 (m, 2 H), 3.55
15 (m, 4 H), 1.62 (m, 6 H); ^{13}C NMR δ 165.8, 144.8, 143.5, 139.6, 133.6, 128.0, 122.9, 60.6, 59.2, 47.9, 42.2, 23.4. Anal. ($\text{C}_{15}\text{H}_{20}\text{Br}_2\text{N}_4\text{O}_6$) C, H, N.

Example 16 (Scheme 2f). 2-((2-Bromoethyl)-2-[[[(2-hydroxypropyl)amino]carbonyl]-4,6-dinitroanilino)ethyl methanesulfonate (IIB-13). A solution of **13** (1.22 g, 4.0 mmol)
20 in 50 mL of CH_2Cl_2 was cooled in an ice-bath, and 3,4-dihydro-2*H*-pyran (1.0 mL) and *p*-toluenesulfonic acid (0.1 g) were added. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**21**) (1.45 g, 94%): as a pale yellow oil; ^1H NMR
25 $[(\text{CD}_3)_2\text{SO}]$ δ 8.99 (d, $J = 2.7$ Hz, 1 H, H-4), 8.81 (m, 1 H, CONH), 8.51 (d, $J = 2.7$ Hz, 1 H, H-6), 4.57 (m, 1 H), 3.72 (m, 2 H), 3.46-3.25 (m, 4 H), 1.82-1.44 (m, 8 H). ^{13}C NMR δ 162.7, 148.4, 145.9, 140.3, 128.2, 125.8, 120.5, 98.0, 64.2, 61.3, 36.5, 30.2, 28.9, 24.9, 19.1. HRMS (FAB) Calcd. For $\text{C}_{15}\text{H}_{19}^{35}\text{ClN}_3\text{O}_7$ $[\text{M}+\text{H}^+]$ m/z 388.0912. Found; 388.0915.

30 Reaction of **21** (1.45 g, 3.75 mmol) with diethanolamine (1.67 g) as above gave 2-[bis(2-hydroxyethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**22**) (1.62 g, 95%): as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.96 (m, 1H, CONH), 8.66

(d, $J = 2.8$ Hz, 1H, H-4), 8.31 (d, $J = 2.8$ Hz, 1H, H-6), 4.95 (m, 2H), 4.56 (m, 1H), 3.79-3.16 (m, 14H), 1.80-1.45 (m, 8 H); ^{13}C NMR δ 166.2, 148.1, 143.6, 139.3, 133.8, 128.9, 123.8, 98.5, 64.8, 61.7, 58.5, 54.6, 37.3, 30.6, 29.2, 25.4, 19.6. HRMS (FAB) Calcd. For $\text{C}_{19}\text{H}_{29}\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 457.1935. Found; 457.1939.

5

Reaction of **22** (1.62 g, 3.55 mmol) with MsCl (2 mL) as above gave 2-[[2-[(methylsulfonyl)oxy]ethyl]-4,6-dinitro-6-({[2-(tetrahydro-2H-pyran-2-yloxy)propyl]-amino}carbonyl)anilino]ethyl methanesulfonate (**23**) (2.17 g, 100%): as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.71 (d, $J = 2.8$ Hz, 1H), 8.71 (m, 1H), 8.31 (d, $J = 2.8$ Hz, 1H), 4.26 (m, 4 H), 3.71-3.37 (m, 10 H), 3.13 (s, 6 H), 3.10 (m, 2 H), 1.82-1.43 (m, 8 H); ^{13}C NMR δ 165.1, 146.3, 145.4, 140.9, 135.9, 127.4, 122.2, 98.0, 67.2, 64.3, 51.4, 45.7, 36.5, 30.2, 28.7, 24.9, 19.1, 8.5. HRMS (FAB) Calcd. For $\text{C}_{21}\text{H}_{33}\text{N}_4\text{O}_{13}\text{S}_2$ $[\text{M}+\text{H}^+]$ m/z 613.1486. Found; 613.1481.

- 15 A solution of **23** (2.95 g, 3.55 mmol) in THF (120 mL) was treated with 1 N HCl (80 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. NaHCO_3 , and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(100:1),
 20 to give 2-(2-[[3-(3-hydroxypropyl)amino]carbonyl]-2-[(methylsulfonyl)oxy]ethyl)-4,6-dinitroanilino)ethyl methanesulfonate (**24**) (1.4 g, 75%): as a yellow solid: mp (EtOAc/petroleum ether) 130-133 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1H), 8.72 (m, 1H), 8.32 (d, $J = 2.8$ Hz, 1H), 4.29 (m, 4 H), 3.47 (m, 8 H), 3.14 (s, 6 H), 1.71 (m, 2 H); ^{13}C NMR δ 165.2, 146.3, 145.3, 140.8, 135.9, 127.5, 122.3, 67.3, 58.4, 51.4,
 25 36.8, 36.5, 31.7. Anal. ($\text{C}_{16}\text{H}_{24}\text{N}_4\text{O}_{12}\text{S}_2$) C, H, N.

Treatment of **24** (0.25 g, 0.45 mmol) with LiBr (53 mg, 0.61 mmol) in EtOAc (50 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **IIB-13** (0.16 g, 66%): as yellow foam; ^1H
 30 NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1H), 8.73 (m, 1H), 8.31 (d, $J = 2.8$ Hz, 1H), 4.28 (m, 2 H), 3.65-3.44 (m, 10 H), 3.13 (s, 3 H), 1.70 (m, 2 H); ^{13}C NMR δ 165.1, 145.7,

145.4, 141.0, 136.2, 127.3, 122.1, 67.5, 58.4, 51.1, 36.7, 36.5, 31.7, 29.6. HRMS (FAB)
Calcd. For $C_{15}H_{22}^{79}BrN_4O_9S$ $[M+H]^+$ m/z 513.0291. Found; 513.0281.

Example 17 (Scheme 2g). 2-((2-bromoethyl)-2-((2-hydroxyethyl)amino)carbonyl)-
5 **4,6-dinitroanilino)ethyl methanesulfonate (IIb-12).** Solid **IIb-7** (300 mg, 0.62 mmol)
and silver methanesulfonate (130 mg, 0.65 mmol) in dry MeCN (15 mL) were heated
under reflux for 3 h, then cooled and filtered. The solid AgBr was washed with EtOAc to
give a 98% yield of AgBr. The solvent was removed at reduced pressure and the residue
was separated by chromatography on silica gel, eluting with EtOAc/petroleum ether (from
10 1:1 to 1:0), to give successively:
- starting material (**IIb-7**) (28 mg, 9%).
- **IIb-12** (123 mg, 38%) as a yellow foam; 1H NMR $[(CD_3)_2SO]$ δ 8.77 (m, 1 H, CONH),
8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.36 (d, $J = 2.7$ Hz, 1 H, H-6), 4.28 (m, 2 H, CH_2OMs),
3.58 (m, 4 H), 3.44 (m, 4 H), 3.14 (s, 3 H, OSO_2CH_3); ^{13}C NMR δ 165.3, 145.8, 145.2,
15 140.9, 135.1, 127.5, 122.2, 67.5, 59.2, 54.2, 51.0, 42.1, 36.4, 29.7; HRMS m/z required
for $C_{14}H_{20}^{79}BrN_4O_9S$ 499.01344; Found 499.01324.

The column was finally eluted with EtOAc/MeOH (9:1) to give 2-(2-((2-
**hydroxyethyl)amino)carbonyl}{2-((methylsulfonyl)oxy)ethyl}-4,6-
dinitroanilino)ethyl methanesulfonate 25 (159 mg, 53%) as a yellow solid: mp 128-132
20 $^{\circ}C$ (EtOAc/petroleum ether); 1H NMR $[(CD_3)_2SO]$ δ 8.78 (m, 1 H, CONH), 8.74 (d, $J =$
2.7 Hz, 1 H, H-4), 8.36 (d, $J = 2.7$ Hz, 1 H, H-6), 4.29 (m, 4 H, $2 \times CH_2OMs$), 3.56 (m, 2
H), 3.45 (m, 6 H), 3.14 (s, 6 H, $2 \times OSO_2CH_3$); ^{13}C NMR δ 165.4, 146.3, 145.1, 140.6,
135.8, 127.6, 122.3, 67.3, 59.2, 51.3, 42.1, 36.4; HRMS: $C_{15}H_{23}N_4O_{12}S_2$ requires m/z
515.0754. Found: 515.0744.**

25

Example 18 (Scheme 2h). 2-((2-Chloroethyl)-2-((2-hydroxyethyl)amino)carbonyl)-
4,6-dinitroanilino)ethyl methanesulfonate (IIb-2m). A solution of **25** (5.3 g, 10.3
mmol) in DMF (100 mL) was treated with LiCl (524 mg, 12.4 mmol) at 60 $^{\circ}C$ for 2 h,
and then cooled, poured into dilute HCl and extracted with EtOAc (3x150 mL). Workup
30 and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether
from 1:1 to 1:0, gave **IIb-2** (2.4 g, 59%), and then **IIb-2m** (1.94 g, 41%) as yellow oil; 1H
NMR $[(CD_3)_2SO]$ δ 8.77 (m, 1H, CONH), 8.74 (d, $J = 2.7$ Hz, 1H, H-4), 8.36 (d, $J = 2.7$

Hz, 1H, H-6), 4.28 (m, 2H, $-\text{CH}_2\text{O}-\text{Ms}$), 3.58 (m, 4H), 3.44 (m, 4H), 3.14 (s, 3 H, $-\text{OSO}_2\text{CH}_3$); ^{13}C NMR δ 165.3, 145.8, 145.2, 140.9, 135.1, 127.5, 122.2, 67.5, 59.2, 54.2, 51.0, 42.1, 36.4, 29.7.

IIb-2m was prepared by an alternative method as following: A solution of **IIb-2** (12.50 g, 31.6 mmol) in 3-methyl-2-butanone (150 mL) was treated at 25 °C with NaI (5.69 g, 38.0 mmol) and the mixture was stirred at 70 °C for 2 h and then concentrated under reduced pressure. The residue was partitioned between water (250 mL) and EtOAc (250 mL) and the separated organic layer was washed with water, dried (Na_2SO_4) and then concentrated under reduced pressure. The resulting oil (15.23 g) was dissolved in CH_3CN (80 mL), treated with silver methanesulfonate (9.63 g, 47.4 mmol) and the mixture was stirred at 25 °C for 1 h and then concentrated under reduced pressure. The residue was extracted with EtOAc (200 mL), filtered, the solids were washed with EtOAc (100 mL) and the EtOAc solution was evaporated and the oily mixture was separated by chromatography on silica gel as above and gave starting material (3.61 g, 29%), **IIb-2m** (4.55 g, 32%) and **25** (4.98 g, 31%). When NaI was replaced by LiBr the reaction gave a similar result.

Example 19 (Scheme 2i). 2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-14) and 2-((2-iodoethyl)-2-((2-hydroxyethyl)amino)carbonyl)-4,6-dinitroanilino)ethyl methanesulfonate (IIb-15).

Treatment of **25** (6.7 g, 13.0 mmol) with NaI (2.9 g, 20 mmol) in EtOAc (200 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **IIb-14** (3.3 g, 44%) as a yellow solid: mp (EtOAc/petroleum ether) 129-131 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.72 (d, $J = 2.8$ Hz, 1 H, H-4), 8.70 (m, 1 H, CONH), 8.32 (d, $J = 2.8$ Hz, 1 H, H-6), 4.80 (m, 1 H), 3.55 (m, 2 H), 3.43 (m, 4 H), 3.31 (m, 6 H); ^{13}C NMR δ 165.3, 145.2, 144.7, 141.0, 136.3, 127.3, 122.0, 59.3, 54.7, 42.1, 2.94. Anal ($\text{C}_{13}\text{H}_{16}\text{N}_4\text{I}_2\text{O}_6$) C, H, N.

Later eluates gave **IIb-15** (1.35 g, 19%) as a yellow foam; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.74 (d, $J = 2.8$ Hz, 1H, H-4), 8.74 (m, 1H, CONH), 8.34 (d, $J = 2.8$ Hz, 1 H, H-6), 4.28 (m, 2 H), 3.56 (m, 2 H), 3.43 (m, 2 H), 3.31 (m, 6 H), 3.13 (s, 3 H); ^{13}C NMR δ 165.3, 145.5, 145.2, 140.8, 136.1, 127.4, 122.1, 67.5, 59.2, 55.4, 50.6, 42.1, 36.5, 2.6. HRMS (FAB) Calcd. For $\text{C}_{14}\text{H}_{20}\text{N}_4\text{O}_9\text{S}$ [$\text{M}+\text{H}^+$] m/z 546.9996. Found; 546.9997.

Example 20 (Scheme 2j). 3-[Bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-2,6-dinitrobenzamide (**IIc-7**) and 2-((2-bromoethyl)-3-((2-hydroxyethyl)amino)carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (**IIc-12**).

5 Treatment of 2-(3-((2-hydroxyethyl)amino)carbonyl)-2-((methylsulfonyl)oxy)ethyl)-2,4-dinitroanilino)ethyl methanesulfonate (**26**) [for method of preparation see NZ Application No. 521851] (310 mg, 0.6 mmol) in EtOAc (50 mL) with LiBr (78 mg, 0.9 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc-7** (70 mg, 25%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.80 (m, 1H, CONH), 8.24 (d, *J* = 9.4 Hz, 1H), 7.63 (d, *J* = 9.4 Hz, 1H), 4.66 (m, 1 H), 3.70 (m, 4 H), 3.60 (m, 4 H), 3.45 (m, 2 H), 3.22 (m, 2 H); ¹³C NMR δ 161.4, 145.8, 140.2, 137.5, 129.2, 127.6, 122.6, 59.0, 52.6, 41.7, 30.0. HRMS (FAB) Calcd. For C₁₃H₁₇⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 482.9515. Found; 482.9508.

15 Further elution with EtOAc/MeOH (50:2) gave **IIc-12** (118 mg, 39%): mp. 94-97 °C; ¹H NMR [(CD₃)₂SO] δ 8.80 (m, 1H, CONH), 8.25 (d, *J* = 9.4 Hz, 1H), 7.64 (d, *J* = 9.4 Hz, 1H), 4.67 (m, 1 H), 4.27 (m, 2 H), 3.63 (m, 4 H), 3.57 (m, 2 H), 3.45 (m, 2 H), 3.26 (m, 2 H), 3.15 (s, 3 H); ¹³C NMR δ 161.4, 146.2, 140.5, 137.7, 129.2, 127.5, 122.9, 66.8, 59.0, 50.0, 41.7, 36.6, 29.9. Anal. (C₁₄H₁₉BrN₄O₉S) C, H, N.

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Example 21 (Scheme 2j). 3-[Bis(2-bromoethyl)amino]-*N*-(3-hydroxypropyl)-2,6-dinitrobenzamide (**IIc-8**) and 2-((2-bromoethyl)-3-((3-hydroxypropyl)amino)carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (**IIc-13**). Treatment of 2-(3-((3-hydroxypropyl)amino)carbonyl)-2-

25 [(methylsulfonyl)oxy)ethyl)-2,4-dinitroanilino)ethyl methanesulfonate (**27**) [for method of preparation see co-pending NZ Application No. 521851] (716 mg, 1.36 mmol) in EtOAc (200 mL) with LiBr (175 mg, 2.0 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc-8** (289 mg, 42%) as a yellow solid; mp (EtOAc/petroleum ether) 142-144 °C; ¹H NMR [(CD₃)₂SO] δ 8.75
30 (t, *J* = 5.8 Hz, 1 H, CONH), 8.23 (d, *J* = 9.4 Hz, 1 H, H-5), 7.62 (d, *J* = 9.4 Hz, 1 H, H-6), 4.47 (m, 1 H, CHOH), 3.68 (m, 4 H), 3.57 (m, 4 H), 3.43 (m, 2 H), 3.20 (m, 2 H), 1.60 (m, 2 H); ¹³C NMR δ 161.2, 146.9, 140.2, 137.5, 129.4, 127.7, 122.6, 58.3, 52.6, 36.4,

31.6, 30.1. HRMS (FAB) Calcd. For $C_{14}H_{19}^{79}Br_2N_4O_6$ $[M+H]^+$ m/z 496.9671. Found: 496.9667.

Further elution with EtOAc/MeOH (50:2) gave **IIc-13** (270 mg, 39%): mp. 115-117 °C;

5 1H NMR $[(CD_3)_2SO]$ δ 8.75 (t, $J = 5.8$ Hz, 1 H, CONH), 8.24 (d, $J = 9.4$ Hz, 1 H, H-5), 7.64 (d, $J = 9.4$ Hz, 1 H, H-6), 4.43 (m, 1 H, CHOH), 4.27 (m, 2 H, CH_2OMs), 3.66 (m, 4 H, $2 \times CH_2N$), 3.59 (m, 2 H), 3.44 (m, 2 H), 3.22 (m, 2 H), 3.15 (s, 3 H, CH_3SO_3), 1.60 (m, 2 H); ^{13}C NMR δ 161.1, 146.2, 140.5, 137.7, 129.2, 127.6, 122.9, 66.8, 58.2, 52.9, 50.0, 36.6, 36.4, 31.6, 30.0. Anal. ($C_{15}H_{21}BrN_4O_9S$) C, H, N.

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Example 22 (Scheme 2j). 3-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-2,6-dinitrobenzamide (**IIc-9**) and 2-((2-bromoethyl)-3-((4-hydroxybutyl)amino)carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (**IIc-14**).

Treatment of 2-(3-((4-hydroxybutyl)amino)carbonyl){2-[(methanesulfonyl)oxy]ethyl}-

15 **2,4-dinitroanilino)ethyl methanesulfonate (28)** [for method of preparation see NZ Application No. 521851] (500 mg, 0.92 mmol) in EtOAc (100 mL) with LiBr (110 mg, 1.4 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc-9** (100 mg, 21%) as a foam; 1H NMR $[(CD_3)_2SO]$ δ 8.73 (m, 1H, CONH), 8.25 (d, $J = 9.4$ Hz, 1H), 7.63 (d, $J = 9.4$ Hz, 1H), 4.38 (m, 1 H), 3.69 (m, 20 4 H), 3.57 (m, 4 H), 3.40 (m, 2 H), 3.14 (m, 2 H), 1.47 (m, 4 H); ^{13}C NMR δ 161.0, 145.8, 140.2, 137.6, 129.3, 127.6, 122.6, 60.2, 52.6, 30.0, 29.6, 24.8. HRMS (FAB) Calcd. For $C_{15}H_{20}^{79}Br_2N_4O_6$ $[M+H]^+$ m/z 510.9828. Found; 510.9819.

Further elution with EtOAc/MeOH (50:2) gave **IIc-14** (117 mg, 30%): mp. 114-117 °C;

25 1H NMR $[(CD_3)_2SO]$ δ 8.74 (m, 1 H, CONH), 8.25 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.37 (m, 1 H), 4.27 (m, 2 H), 3.65 (m, 4 H), 3.57 (m, 2 H), 3.35 (m, 2 H), 3.16 (m, 2 H), 3.15 (s, 3 H), 1.47 (m, 4 H); ^{13}C NMR δ 160.0, 146.1, 140.6, 137.8, 129.2, 127.5, 122.9, 66.8, 60.2, 52.9, 50.0, 36.6, 29.9, 29.6, 24.9. Anal. ($C_{16}H_{23}BrN_4O_9S$) C, H, N.

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Example 23 (Scheme 2k). 2-(3-[[[(3-hydroxypropyl)amino]carbonyl]{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (27) and 2-((2-bromoethyl)-3-[[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino)ethyl methanesulfonate (**IIc-13**). Solid **IIc-8** (2.15 g, 4.3 mmol) was added to a hot solution of silver methanesulfonate (0.992 g, 4.9 mmol) in dry MeCN (40 mL). The mixture was heated under reflux for 3 h, then cooled and filtered. The solvent was removed at reduced pressure and the residue was separated by chromatography on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 1:0), to give successively **IIc-13** (0.5 g, 25%), **IIc-8** (0.3 g, 14%) and 27 (0.4 g, 18%).

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Example 24 (Scheme 2k). 2-((2-chloroethyl)-3-[[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino)ethyl methanesulfonate (**IIc-6**). A solution of 27 (9.0 g, 17.0 mmol) in DMF (110 mL) was treated with LiCl (860 mg, 20.4 mmol) at 60 °C for 2 h, then cooled, poured into dilute HCl, and extracted with EtOAc (3x150 mL). Workup and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether from 1:1 to 1:0, gave **IIc-6** (4.0 g, 50%) as yellow crystals: mp 104-109°C; ¹H NMR [(CD₃)₂SO] δ 8.75 (t, J = 5.8 Hz, 1H, CONH), 8.24 (d, J = 9.4 Hz, 1H, H-5), 7.64 (d, J = 9.4 Hz, 1H, H-6), 4.44 (m, 1H, CHOH), 4.26 (m, 2H), 3.72 (m, 2H), 3.65 (m, 2H), 3.59 (m, 2H), 3.43 (m, 2H), 3.20 (m, 2H), 3.15 (s, 3H), 1.60 (m, 2H); ¹³C NMR δ 161.1, 146.4, 140.5, 137.7, 129.2, 127.6, 122.9, 66.8, 58.2, 52.9, 50.1, 41.4, 36.6, 36.4, 31.6. Anal. (C₁₅H₂₁ClN₄O₉S) C, H, N.

Example 25 (Scheme 2k). 2-((2-Iodoethyl)-3-[[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino)ethyl methanesulfonate (**IIc-15**). A solution of 27 (5.28 g, 10.0 mmol) in EtOAc (250 mL) was treated with NaI (1.8 g, 12.0 mmol) at 60 °C for 2 h, and the product was chromatographed on silica gel, eluting with EtOAc/petroleum ether from 1:2 to 1:0, to give **IIc-15** (2.29 g, 41%) as yellow crystals: mp 100-103°C; ¹H NMR [(CD₃)₂SO] δ 10.05 (s, 1H), 7.40 (d, J = 11.5 Hz, 1H), 7.09 (s, 1H), 6.70 (d, J = 11.5 Hz, 1H), 2.50 (m, 2H), 2.21 (m, 2H), 2.03 (s, 3H), 1.52 (m, 4H); ¹³C NMR δ 161.1, 145.8, 140.5, 137.7, 129.2, 127.6, 122.9, 66.8, 58.2, 53.9, 49.9, 41.4, 36.6, 36.4, 31.6. Anal. (C₁₅H₂₁I N₄O₉S) C, H, N.

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Preparation of Phosphates (Scheme 3)

Example 26. 2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-7P). A solution of alcohol Ib-7 (2.58 g, 5.33 mmol) and di-
5 *tert*-butyl diethylphosphoramidite (93%, 2.0 mL, 6.9 mmol) in dry DMF (20 mL) under N₂ was treated with 1*H*-tetrazole (3 wt. % in CH₃CN, 55 mL, 18.7 mmol) and stirred at 20 °C for 1.5 h. The reaction mixture was then cooled to -50 °C and a solution of 3-chloroperoxybenzoic acid (55%, 2.68 g, 8.54 mmol) was rapidly added such that the temperature was kept below -5 °C. The reaction mixture was warmed to room
10 temperature and diluted with CH₂Cl₂ (150 mL). The solution was washed with 5% aqueous Na₂S₂O₅ (2 x 50 mL), 10% aqueous NaHCO₃ (2 x 50 mL), water (2 x 50 mL), dried, concentrated under reduced pressure below 30 °C and the residue was shaken with *i*-Pr₂O/hexane and refrigerated. The resulting solid was purified by chromatography on silica gel, eluting with CH₂Cl₂/EtOAc, followed by recrystallisation from CH₂Cl₂/hexane
15 (below 40 °C) to give di-*tert*-butyl 2-[[2-[bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate (Ib-7E) (2.59 g, 72%) as an unstable yellow solid: mp 99-101 °C (dec); ¹H NMR [(CD₃)₂SO] δ 8.93 (t, *J* = 5.6 Hz, 1 H), 8.76 (d, *J* = 2.8 Hz, 1 H), 8.33 (d, *J* = 2.8 Hz, 1 H), 4.01 (g, *J* = 6.1 Hz, 2 H), 3.62-3.42 (m, 10 H), 1.43 (s, 18 H). HRMS (FAB) calcd for C₂₁H₃₄⁷⁹Br₂N₄O₉P (MH⁺) *m/z* 675.0430 found 675.0398;
20 calcd for C₂₁H₃₄⁷⁹Br⁸¹BrN₄O₉P (MH⁺) *m/z* 677.0410, found 677.0397; calcd for C₂₁H₃₄⁸¹Br₂N₄O₉P (MH⁺) *m/z* 679.0389, found 679.0398. Anal. (C₂₁H₃₃Br₂N₄O₉P).

A solution of Ib-7E (2.80 g, 4.14 mmol) and TFA (15 mL) in dry CH₂Cl₂ (15 mL) was stirred at 20 °C for 1 h, then concentrated under reduced pressure. Residual TFA was
25 removed azeotropically with CH₃CN (2 x) and the resulting residue was dissolved in EtOAc. Addition of excess hexane precipitated a semisolid which was dried under high vacuum at 20 °C to give Ib-7P (98%) as a yellow foam. ¹H NMR [(CD₃)₂SO] δ 8.93 (t, *J* = 5.6 Hz, 1 H), 8.75 (d, *J* = 2.8 Hz, 1 H), 8.36 (d, *J* = 2.8 Hz, 1 H), 3.97 (q, *J* = 6.3 Hz, 2 H), 3.62-3.43 (m, 10 H). HRMS (FAB) calcd for C₁₃H₁₈⁷⁹Br₂N₄O₉P (MH⁺) *m/z* 562.9178,
30 found 562.9171; calcd for C₁₃H₁₈⁷⁹Br⁸¹BrN₄O₉P (MH⁺) *m/z* 564.9158, found 564.9152; calcd for C₁₃H₁₈⁸¹Br₂N₄O₉P (MH⁺) *m/z* 566.9137, found 566.9121. Treatment of diacid Ib-7P with NaHCO₃ (2.0 equiv.) gave the disodium salt.

Example 27. 3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate (**Ia-3P**). Similar phosphorylation of **IIa-3**, followed by chromatography of the product on silica gel and elution with CH₂Cl₂/EtOAc (2:3), gave di-*tert*-butyl 3-[[5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl phosphate (**Ia-3E**) (76%) as a yellow solid: mp (EtOAc/*i*-Pr₂O/hexane) 120-121°C (dec); ¹H NMR [(CD₃)₂SO] δ 8.70 (t, *J* = 5.6 Hz, 1 H), 8.55 (s, 1 H), 7.45 (s, 1 H), 3.96 (q, *J* = 6.7 Hz, 2 H), 3.82 (t, *J* = 5.8 Hz, 4 H), 3.69 (t, *J* = 5.8 Hz, 4 H), 3.34 (after D₂O exchange, t, *J* = 6.8 Hz, 2 H), 1.86 (pent, *J* = 6.6 Hz, 2 H), 1.42 (s, 18 H). Anal. (C₂₂H₃₅Cl₂N₄O₉P) C, H, N.

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Similar treatment of ester **Ia-3E** with TFA gave diacid **Ia-3P** (99%) as a hygroscopic yellow solid. ¹H NMR [(CD₃)₂SO] δ 8.71 (t, *J* = 5.6 Hz, 1 H), 8.54 (s, 1 H), 7.45 (s, 1 H), 3.92 (q, *J* = 6.7 Hz, 2 H), 3.82 (t, *J* = 5.8 Hz, 4 H), 3.69 (t, *J* = 5.8 Hz, 4 H), 3.31 (q, *J* = 6.5 Hz, 2 H), 1.84 (pent, *J* = 6.6 Hz, 2 H). HRMS (FAB) Calcd. for C₁₄H₂₀³⁵Cl₂N₄O₉P [M+H]⁺ *m/z* 489.0345; found 489.0344. Calcd. for C₁₄H₂₀³⁵Cl³⁷ClN₄O₉P [M+H]⁺ *m/z* 491.0316; found 491.0317. Calcd. for C₁₄H₂₀³⁷Cl₂N₄O₉P [M+H]⁺ *m/z* 493.0286; found 493.0312. Treatment of diacid **I-3P** with NaHCO₃ (2:0 equiv) gave the disodium salt.

Example 28. 3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate (**Ia-8P**). Similar phosphorylation of **IIa-8**, followed by chromatography of the product on silica gel and elution with CH₂Cl₂/EtOAc (1:1), gave di-*tert*-butyl 3-[[5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl phosphate (**Ia-8E**) (66%) as a yellow solid: mp (EtOAc/*i*-Pr₂O/hexane) 110-111°C (dec). ¹H NMR ((CD₃)₂SO) δ 8.70 (t, *J* = 5.6 Hz, 1 H), 8.55 (s, 1 H), 7.44 (s, 1 H), 3.96 (q, *J* = 6.7 Hz, 2 H), 3.79-3.63 (m, 8 H), 3.35 (after D₂O exchange, t, *J* = 6.8 Hz, 2 H), 1.86 (pent, *J* = 6.6 Hz, 2 H), 1.42 (s, 18 H). Anal. (C₂₂H₃₅Br₂N₄O₉P) C, H, N.

Similar treatment of ester **Ia-8E** with TFA gave diacid **Ia-8P** (99%) as a hygroscopic yellow solid. ¹H NMR [(CD₃)₂SO] δ 8.71 (t, *J* = 5.6 Hz, 1 H), 8.55 (s, 1 H), 7.43 (s, 1 H), 3.93 (q, *J* = 6.7 Hz, 2 H), 3.79-3.63 (m, 8 H), 3.31 (q, *J* = 6.5 Hz, 2 H), 1.85 (pent, *J* = 6.6 Hz, 2 H). HRMS (FAB) calcd for C₁₄H₂₀⁷⁹Br₂N₄O₉P (MH⁺) *m/z* 576.9335, found 576.9314; calcd for C₁₄H₂₀⁷⁹Br⁸¹BrN₄O₉P (MH⁺) *m/z* 578.9314, found 578.9305; calcd

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for $C_{14}H_{20}^{81}Br_2N_4O_9P$ (MH^+) m/z 580.9294, found 580.9297. Treatment of diacid **Ia-8P** with $NaHCO_3$ (2.0 equiv.) gave the disodium salt.

Example 29. 2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-2P). Similar phosphorylation of **Ib-2**, followed by chromatography of the product on silica gel and elution with $CH_2Cl_2/EtOAc$ (13:7), gave di-*tert*-butyl 2-[[2-[bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate (**Ib-2E**) (72%) as a yellow solid: mp ($EtOAc/i-Pr_2O$ /hexane) 107-108 °C (dec); 1H NMR [$(CD_3)_2SO$] δ 8.90 (t, $J = 5.6$ Hz, 1 H), 8.75 (d, $J = 2.8$ Hz, 1 H), 8.33 (d, $J = 2.8$ Hz, 1 H), 4.01 (q, $J = 6.1$ Hz, 2 H), 3.72 (t, $J = 6.8$ Hz, 4 H), 3.53 (q, $J = 5.5$ Hz, 2 H), 3.43 (t, $J = 6.8$ Hz, 4 H), 1.43 (s, 18 H). Anal. ($C_{21}H_{33}Cl_2N_4O_9P$) C, H, N, P. CRL 11363.

Similar treatment of ester **Ib-2E** with TFA gave diacid **Ib-2P** (98%) as a yellow foam. 1H NMR [$(CD_3)_2SO$] δ 8.89 (t, $J = 5.6$ Hz, 1 H), 8.74 (d, $J = 2.8$ Hz, 1 H), 8.36 (d, $J = 2.8$ Hz, 1 H), 3.98 (q, $J = 6.2$ Hz, 2 H), 3.72 (t, $J = 6.7$ Hz, 4 H), 3.51 (q, $J = 5.6$ Hz, 2 H), 3.43 (t, $J = 6.7$ Hz, 4 H). HRMS (FAB) Calcd. for $C_{13}H_{18}^{35}Cl_2N_2O_9P$ $[M+H]^+$ m/z 475.0189; found 475.0189. Calcd. for $C_{13}H_{18}^{35}Cl^{37}ClN_2O_9P$ $[M+H]^+$ m/z 477.0159; found 477.0167. Calcd. for $C_{13}H_{18}^{35}Cl_2N_2O_9P$ $[M+H]^+$ m/z 479.0130; found 479.0160. Treatment of diacid **Ib-2P** with $NaHCO_3$ (1.0 equiv.) gave the monosodium salt.

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Example 30. 2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonoxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ib-2mP). Similar phosphorylation of **Ib-2m**, followed by chromatography of the product on silica gel and elution with $EtOAc$, gave 2-[(2-chloroethyl)-2-(6-*tert*-butoxy-8,8-dimethyl-6-oxido-5,7-dioxo-2-aza-6-phosphanon-1-onyl)-4,6-dinitroanilino]ethyl methanesulfonate (**Ib-2mE**) (80%) as a yellow foam. 1H NMR [$(CD_3)_2SO$] δ 8.94 (t, $J = 5.6$ Hz, 1 H), 8.75 (d, $J = 2.8$ Hz, 1 H), 8.34 (d, $J = 2.8$ Hz, 1 H), 4.28 (t, $J = 5.4$ Hz, 2 H), 4.02 (q, $J = 6.2$ Hz, 2 H), 3.74-3.43 (m, 8 H), 3.13 (s, 3 H), 1.43 (s, 18 H). ^{13}C NMR δ 265.6, 146.2, 145.3, 140.8, 135.6, 127.5, 122.4, 81.7, 67.5, 64.2, 54.3, 51.3, 41.4, 36.5, 29.5.

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Similar treatment of ester **Ib-2mE** with TFA gave diacid **Ib-2mP** (68%) as a yellow solid. Mp ($EtOAc/CH_2Cl_2$): 132-134 °C; 1H NMR [$(CD_3)_2SO$] δ 8.92 (t, $J = 5.6$ Hz, 1 H), 8.74

(d, $J = 2.8$ Hz, 1 H), 8.37 (d, $J = 2.8$ Hz, 1 H), 4.29 (t, $J = 5.4$ Hz, 2 H), 3.98 (q, $J = 6.0$ Hz, 2 H), 3.58-3.40 (after D₂O exchange, m, 8 H), 3.13 (s, 2 H). ¹³C NMR δ 165.5, 146.1, 145.3, 140.8, 135.7, 127.6, 122.3, 67.5, 63.3, 63.2, 54.3, 51.3, 41.3, 36.5. Anal. (C₁₄H₂₀ClN₄O₁₂PS) C, H, N.

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Example 31. 2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl}amino)ethyl dihydrogen phosphate (Ib-7aP). Similar phosphorylation of alcohol **Ib-7a** (0.67 g, 1.3 mmol) with di-*tert*-butyl diethylphosphoramidite (93%, 489 mg, 2.0 mmol), followed by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1) gave **Ib-7aE** as a yellow solid (0.74 g, 81%): mp (EtOAc/petroleum ether) 121-123 °C; ¹H NMR [(CD₃)₂SO] δ 9.09 (m, 1 H), 8.73 (m, 1 H), 8.32 (m, 1 H), 4.44 (m, 2 H), 4.00 (m, 2 H), 3.39 (m, 2 H), 3.60 (m, 4 H), 1.62 (m, 6 H), 1.44 (s, 18 H). ¹³C NMR δ 165.9, 144.8, 143.6, 139.6, 133.2, 128.0, 123.1, 81.6, 64.0, 60.4, 39.9, 29.4, 23.5. Anal. (C₂₃H₃₇Br₂N₄O₉P) C, H, N.

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Similar treatment of **Ib-7aE** (100 mg) with TFA (6 mL), followed by crystallization from CH₂Cl₂/EtOAc, gave **Ib-7aP** as a yellow solid (70 mg, 85%): mp 157-161 °C; ¹H NMR [(CD₃)₂SO] δ 9.07 (m, 1 H), 8.72 (m, 1 H), 8.36 (m, 1 H), 4.43 (m, 2 H), 4.00 (m, 2 H), 3.52 (m, 6 H), 1.62 (m, 6 H). ¹³C NMR δ 165.9, 144.8, 143.6, 139.7, 133.4, 128.1, 123.1, 63.2, 60.4, 47.9, 39.9, 23.5. Anal. (C₁₅H₂₁Br₂N₄O₉P) C, H, N.

20

Example 32. 2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonoxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ib-12P). Similar phosphorylation of **Ia-12**, followed by chromatography of the product on silica gel and elution with EtOAc, gave 2-[(2-bromoethyl)-2-(6-*tert*-butoxy-8,8-dimethyl-6-oxido-5,7-dioxo-2-aza-6-phosphanon-1-anoyl)-4,6-dinitroanilino]ethyl methanesulfonate (**Ib-12E**) (66%) as a yellow foam. ¹H NMR [(CD₃)₂SO] δ 8.94 (t, $J = 5.6$ Hz, 1 H), 8.75 (d, $J = 2.8$ Hz, 1 H), 8.34 (d, $J = 2.8$ Hz, 1 H), 4.28 (t, $J = 5.4$ Hz, 2 H), 4.02 (q, $J = 6.2$ Hz, 2 H), 3.62-3.43 (m, 8 H), 3.13 (s, 3 H), 1.43 (s, 18 H). HRMS (FAB) calcd for C₂₂H₃₇⁷⁹BrN₄O₁₂PS [M+H]⁺ m/z 693.1029; found 693.1010.

30

Similar treatment of ester **Ib-12E** with TFA gave diacid **Ib-12P** (98%) as a yellow foam.
¹H NMR [(CD₃)₂SO] δ 8.92 (t, *J* = 5.6 Hz, 1 H), 8.74 (d, *J* = 2.8 Hz, 1 H), 8.37 (d, *J* = 2.8 Hz, 1 H), 4.28 (t, *J* = 5.4 Hz, 2 H), 3.98 (q, *J* = 6.0 Hz, 2 H), 3.58-3.40 (after D₂O exchange, m, 8 H), 3.13 (s, 2 H). HRMS (FAB) calcd for C₁₄H₂₁⁷⁹BrN₄O₁₂PS [M+H]⁺
 5 *m/z* 578.9798; found 578.9784; calcd for C₁₄H₂₁⁸¹Br⁸¹BrN₄O₁₂PS [M+H]⁺ *m/z* 580.9777; found 580.9784. Treatment of diacid **Ib-12P** with NaHCO₃ (1.0 equiv) gave the monosodium salt.

Example 33. 2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-14P). Similar phosphorylation of **Ib-14**, followed by chromatography of the product on silica gel and elution with CH₂Cl₂/EtOAc (3:1), gave di-*tert*-butyl 2-[[2-[bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate (**Ib-14E**) (67%) as a yellow solid: mp (CH₂Cl₂/i-Pr₂O/hexane) 108-110 °C (dec); ¹H NMR [(CD₃)₂SO] δ 8.91 (t, *J* = 5.6 Hz, 1 H), 8.74 (d, *J* = 2.8 Hz, 1 H), 8.30 (d, *J* = 2.8 Hz, 1 H),
 15 4.01 (q, *J* = 6.3 Hz, 2 H), 3.53 (q, *J* = 5.7 Hz, 2 H), 3.45 (t, *J* = 7.8 Hz, 4 H), 3.24 (after D₂O exchange, t, *J* = 7.6 Hz, 4 H), 1.44 (s, 18 H). Anal. (C₂₁H₃₃I₂N₄O₉P), C, H, N, P.

Similar treatment of ester **Ib-14E** with TFA gave diacid **Ib-14P** (97%) as a yellow foam.
¹H NMR [(CD₃)₂SO] δ 8.90 (t, *J* = 5.6 Hz, 1 H), 8.73 (d, *J* = 2.8 Hz, 1 H), 8.34 (d, *J* = 2.8 Hz, 1 H), 3.98 (q, *J* = 6.4 Hz, 2 H), 3.49 (after D₂O exchange t, *J* = 5.6 Hz, 2 H), 3.45 (t, *J* = 7.8 Hz, 4 H), 3.29 (t, *J* = 7.7 Hz, 4 H). HRMS (FAB) Calcd. for C₁₃H₁₈I₂N₄O₉ [M+H]⁺
 20 *m/z* 658.3911; found 658.3907. Treatment of diacid **Ib-14P** with NaHCO₃ (2.0 equiv.) gave the disodium salt.

25 **Example 34. 2-[(2-Iodoethyl)-2,4-dinitro-6-({2-(phosphonoxy)ethyl}amino)carbonyl]-anilino]ethyl methanesulfonate (Ib-15P).**
 Similar phosphorylation of alcohol **Ib-15** (1.68 g, 3.1 mmol) with di-*tert*-butyl diethylphosphoramidite (93%, 1.15 g, 4.5 mmol), followed by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1), and
 30 crystallization from EtOAc/petroleum ether, gave **Ib-15E** as a yellow solid (2.23 g, 97%): mp (EtOAc/petroleum ether) 109-111 °C; ¹H NMR [(CD₃)₂SO] δ 8.98 (m, 1 H), 8.76 (d, *J* = 2.8 Hz, 1 H), 8.33 (d, *J* = 2.8 Hz, 1 H), 4.27 (m, 2 H), 4.00 (m, 2 H), 3.53 (m, 2 H), 3.46

(m, 4 H), 3.14 (s, 3 H), 1.43 (s, 18 H). ^{13}C NMR δ 165.5, 145.6, 145.2, 140.8, 135.6, 127.4, 122.4, 81.7, 67.5, 64.2, 55.4, 50.7, 39.9, 36.5, 29.3, 2.6. Anal. ($\text{C}_{22}\text{H}_{36}\text{N}_4\text{O}_{12}\text{PS}$), C, H, N.

- 5 Similar treatment of **Ib-15E** (405 mg) with TFA (6 mL) and crystallization of the product from CH_2Cl_2 /petroleum ether gave diacid **Ib-15P** as a yellow solid (306 mg, 89%): mp 147-150 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.93 (m, 1 H), 8.74 (d, $J = 2.8$ Hz, 1 H), 8.36 (d, $J = 2.8$ Hz, 1 H), 4.27 (m, 2 H), 4.00 (m, 2 H), 3.46 (m, 6 H), 3.31 (m, 2 H), 3.12 (s, 3 H). ^{13}C NMR δ 165.5, 145.6, 145.2, 140.8, 135.7, 127.6, 122.3, 67.6, 63.3, 55.5, 50.7, 39.9, 36.5, 2.7. Anal. ($\text{C}_{14}\text{H}_{20}\text{N}_4\text{O}_9\text{PS}$), C, H, N.

Example 35. 2-[(2-Chloroethyl)-2,4-dinitro-3-[[[3-(phosphonoxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ic-6P). Similar phosphorylation of **Ic-6**, followed by chromatography of the product on silica gel and elution with

- 15 EtOAc/petroleum ether (from 1:2 to 1:1), gave 2-[(2-chloroethyl)-3-(7-*tert*-butoxy-9,9-dimethyl-7-oxido-6,8-dioxo-2-aza-7-phosphahex-1-anoyl)-2,4-dinitroanilino]ethyl methanesulfonate (**Ic-6E**) (98%) as a yellow solid: mp (EtOAc/petroleum ether) 98-102 °C. ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.83 (t, $J = 5.6$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.29 (t, $J = 5.3$ Hz, 2 H), 3.92 (q, $J = 6.7$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.62-
20 3.55 (m, 2 H), 3.23 (q, $J = 6.5$ Hz, 2 H), 3.15 (s, 3 H), 1.79 (pent, $J = 6.7$ Hz, 2 H), 1.42 (s, 18 H). ^{13}C NMR δ 161.3, 146.4, 140.4, 137.6, 129.1, 127.6, 123.0, 81.2, 66.8, 64.1, 64.0, 52.9, 50.1, 41.4, 36.6, 35.9, 29.3. Anal. ($\text{C}_{23}\text{H}_{38}\text{ClN}_4\text{O}_{12}\text{PS}$) C, H, N.

Similar treatment of ester **Ic-6E** with TFA gave diacid **Ic-6P** (84%) as a yellow solid: mp

- 25 (EtOAc/ CH_2Cl_2) 98-102 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.84 (t, $J = 5.7$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.28 (t, $J = 5.3$ Hz, 2 H), 3.88 (q, $J = 6.8$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.53 (after D_2O exchange, t, $J = 6.0$ Hz, 2 H), 3.23 (q, $J = 6.6$ Hz, 2 H), 3.15 (s, 3 H), 1.76 (pent, $J = 6.7$ Hz, 2 H). Anal. ($\text{C}_{15}\text{H}_{22}\text{ClN}_4\text{O}_{12}\text{PS}$) C, H, N.

- 30 **Example 36. 3-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl}amino)propyl dihydrogen phosphate (Ic-8P).** Similar phosphorylation of alcohol **Ic-8** (1.41 g, 2.83 mmol) with di-*tert*-butyl diethylphosphoramidite (93%, 1.25 g, 5.0 mmol), followed by

flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1), gave **Ic-8E** as a yellow solid (1.77 g, 91%): mp (EtOAc/petroleum ether) 112-114 °C; ¹H NMR [(CD₃)₂SO] δ 8.86 (m, 1 H), 8.24 (d, *J* = 9.4 Hz, 1 H), 7.63 (d, *J* = 9.4 Hz, 1 H), 3.92 (m, 2 H), 3.70 (m, 4 H), 3.60 (m, 4 H), 3.22 (m, 2 H), 1.78 (m, 2 H), 1.41 (s, 18 H). ¹³C NMR δ 161.4, 145.9, 139.9, 137.3, 129.2, 127.8, 122.5, 81.3, 64.1, 52.5, 35.9, 30.1, 29.4. 29.1. Anal. (C₂₂H₃₅Br₂N₄O₉P), C, H, N.

Similar treatment of **Ic-8E** (900 mg) with TFA (10 mL) gave diacid **Ic-8P** as a yellow foam (754 mg, 100%): ¹H NMR [(CD₃)₂SO] δ 8.83 (m, 1 H), 8.24 (d, *J* = 9.4 Hz, 1 H), 7.63 (d, *J* = 9.4 Hz, 1 H), 3.86 (m, 2 H), 3.73 (m, 4 H), 3.60 (m, 4 H), 3.22 (m, 2 H), 1.76 (m, 2 H). ¹³C NMR δ 161.3, 145.9, 140.1, 137.4, 129.2, 127.6, 122.5, 62.9, 52.5, 36.0, 30.0, 29.3. HRMS (FAB) calcd for C₁₄H₂₀⁷⁹Br₂N₄O₉P. [M+H]⁺ *m/z* 576.9335, found 576.9326.

Example 37. 2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ic-12P). Similar phosphorylation of **Ic-12**, followed by chromatography of the product on silica gel and elution with EtOAc/petroleum ether (from 1:2 to 1:0), gave (**Ic-12E**) (99%) as a yellow solid: mp (EtOAc/petroleum ether) 82-86 °C (dec). ¹H NMR [(CD₃)₂SO] δ 9.00 (t, *J* = 5.6 Hz, 1 H), 8.26 (d, *J* = 9.4 Hz, 1 H), 7.65 (d, *J* = 9.4 Hz, 1 H), 4.28 (t, *J* = 5.3 Hz, 2 H), 3.92 (q, *J* = 6.7 Hz, 2 H), 3.72-3.62 (m, 4 H), 3.62-3.55 (m, 2 H), 3.23 (q, *J* = 6.5 Hz, 2 H), 3.15 (s, 3 H), 1.42 (s, 18 H). Anal. (C₂₂H₃₆BrN₄O₁₂PS) C, H, N.

Similar treatment of ester **Ic-12E** with TFA gave diacid **Ic-12P** (100%) as a yellow solid: mp (EtOAc/CH₂Cl₂) 93-97 °C; ¹H NMR [(CD₃)₂SO] δ 8.99 (t, *J* = 5.7 Hz, 1 H), 8.26 (d, *J* = 9.4 Hz, 1 H), 7.65 (d, *J* = 9.4 Hz, 1 H), 4.28 (t, *J* = 5.3 Hz, 2 H), 3.88 (q, *J* = 6.8 Hz, 2 H), 3.72-3.62 (m, 4 H), 3.53 (after D₂O exchange, t, *J* = 6.0 Hz, 2 H), 3.23 (q, *J* = 6.6 Hz, 2 H), 3.15 (s, 3 H). Anal. (C₁₄H₂₀BrN₄O₁₂PS) C, H, N.

Example 38. 2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ic-13P). Similar phosphorylation of **Ic-13**, followed by chromatography of the product on silica gel and elution with CH₂Cl₂/EtOAc

(1:3), gave 2-[(2-bromoethyl)-3-(7-*tert*-butoxy-9,9-dimethyl-7-oxido-6,8-dioxo-2-aza-7-phosphahex-1-onyl)-2,4-dinitroanilino]ethyl methanesulfonate (**Ic-13E**) (70%) as a yellow solid: mp (CH₂Cl₂/i-Pr₂O) 95-96 °C (dec). ¹H NMR [(CD₃)₂SO] δ 8.83 (t, *J* = 5.6 Hz, 1 H), 8.26 (d, *J* = 9.4 Hz, 1 H), 7.65 (d, *J* = 9.4 Hz, 1 H), 4.28 (t, *J* = 5.3 Hz, 2 H),
5 3.92 (q, *J* = 6.7 Hz, 2 H), 3.72-3.62 (m, 4 H), 3.62-3.55 (m, 2 H), 3.23 (q, *J* = 6.5 Hz, 2 H), 3.15 (s, 3 H), 1.79 (pent, *J* = 6.7 Hz, 2 H), 1.42 (s, 18 H). Anal. (C₂₃H₃₈BrN₄O₁₂PS) C, H, N, P.

Similar treatment of ester **Ic-13E** with TFA gave diacid **Ic-13P** (98%) as a hygroscopic
10 yellow solid. ¹H NMR [(CD₃)₂SO] δ 8.84 (t, *J* = 5.7 Hz, 1 H), 8.26 (d, *J* = 9.4 Hz, 1 H), 7.65 (d, *J* = 9.4 Hz, 1 H), 4.28 (t, *J* = 5.3 Hz, 2 H), 3.88 (q, *J* = 6.8 Hz, 2 H), 3.72-3.62 (m, 4 H), 3.53 (after D₂O exchange, t, *J* = 6.0 Hz, 2 H), 3.23 (q, *J* = 6.6 Hz, 2 H), 3.15 (s, 3 H), 1.76 (pent, *J* = 6.7 Hz, 2 H). HRMS (FAB) calcd for C₁₅H₂₃⁷⁹BrN₄O₁₂PS [M+H]⁺ *m/z* 592.9954; found 592.9956. Treatment of diacid **Ic-13P** with NaHCO₃ (1:0 equiv) gave the
15 monosodium salt.

Example 39. 2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ic-15P**).** Similar phosphorylation of **Ic-15**, followed by chromatography of the product on silica gel and elution with CH₂Cl₂/EtOAc
20 (1:3), gave 2-[(2-iodoethyl)-3-(7-*tert*-butoxy-9,9-dimethyl-7-oxido-6,8-dioxo-2-aza-7-phosphahex-1-onyl)-2,4-dinitroanilino]ethyl methanesulfonate (**Ic-15E**) (58%) as a yellow solid: mp (EtOAc/iPr₂O) 90-100 °C. ¹H NMR [(CD₃)₂SO] δ 8.86 (t, *J* = 5.6 Hz, 1 H), 8.25 (d, *J* = 9.4 Hz, 1 H), 7.63 (d, *J* = 9.5 Hz, 1 H), 4.27 (t, *J* = 5.2 Hz, 2 H), 3.91 (q, *J* = 6.7 Hz, 2 H), 3.67 (t, *J* = 5.2 Hz, 2 H), 3.60 (t, *J* = 7.1 Hz, 2 H), 3.26-3.17 (after D₂O
25 exchange, m, partially obscured, 2 H), 3.23 (q, *J* = 6.5 Hz, 2 H), 3.15 (s, 3 H), 1.78 (pent, *J* = 6.6 Hz, 2 H). Anal. (C₂₃H₃₈IN₄O₁₂PS) C, H, N, P.

Similar treatment of ester **Ic-15E** with TFA gave diacid **Ic-15P** (97%) as a hygroscopic yellow solid: mp (CH₃CN/EtOAc) 84-86 °C. ¹H NMR [(CD₃)₂SO] δ 8.90 (t, *J* = 5.6 Hz, 1
30 H), 8.24 (d, *J* = 9.4 Hz, 1 H), 7.57 (d, *J* = 9.5 Hz, 1 H), 4.25 (t, *J* = 5.2 Hz, 2 H), 3.81 (after D₂O exchange, q, *J* = 6.7 Hz, 2 H), 3.62 (after D₂O exchange, t, *J* = 5.2 Hz, 2 H), 3.56 (t, *J* = 7.1 Hz, 2 H), 3.26 (t, *J* = 6.9 Hz, 2 H), 3.20 (q, *J* = 6.5 Hz, 2 H), 3.09 (s, 3 H),

1.73 (pent, $J = 6.6$ Hz, 2 H). HRMS(FAB) calcd for $C_{15}H_{22}IN_4O_{12}PS$ (MH^+) m/z 640.9816. Found; 640.9795. Anal. ($C_{15}H_{22}IN_4O_{12}PS$) C, H.

Table 2. Combustion analysis data for new compounds of Tables 1a and 1b

No	Found				Calculated			
	C	H	N	other	C	H	N	other
IIa-1	44.5	3.9	18.6		44.6	4.1	18.9	
IIa-3	41.3	4.3	13.7	17.4 (Cl)	41.1	4.4	13.7	17.3 (Cl)
IIa-7	32.6	3.3	11.6	33.3 (Br)	32.3	3.3	11.6	33.0 (Br)
IIa-7s	33.4	3.7	7.8		32.5	3.7	8.1	
IIa-8	33.9	3.6	11.4	32.1 (Br)	33.8	3.6	11.3	32.1 (Br)
IIa-9	35.5	3.8	10.7	31.2 (Br)	35.2	3.9	10.9	31.2 (Br)
IIa-14	27.3	2.6	9.6	43.8 (I)	27.0	2.8	9.7	43.9 (I)
IIb-1	51.2	5.7	15.9		51.3	5.7	15.9	
IIb-3	41.6	4.5	13.6	17.1 (Cl)	41.1	4.4	13.7	17.3 (Cl)
IIb-7	32.9	3.3	11.5	33.3 (Br)	32.3	3.3	11.6	33.0 (Br)
IIb-7a	35.3	3.8	10.9		35.2	3.9	10.9	
IIb-8	34.9	3.7	11.3	32.3 (Br)	33.8	3.6	11.3	33.3 (Br)
IIb-14	27.8	3.1	9.5		27.0	2.8	9.7	
IIc-12	33.8	3.7	11.0		33.7	3.8	11.2	
IIc-13	35.4	3.9	11.0		35.2	4.1	10.9	
IIc-14	36.7	4.5	10.2		36.4	4.4	10.6	
Ib-7E	37.7	4.9	8.3	4.6 (P)	37.3	4.9	8.3	4.6 (P)
Ib-2E	44.8	6.2	9.0	5.1 (P)				
Ib-14E	32.9	4.2	7.2	3.8 (P)	32.7	4.3	7.3	4.0 (P)
Ia-3E	44.2	5.9	9.3		43.9	5.9	9.3	
Ia-8E	38.5	5.0	8.2		38.3	5.1	8.1	
Ic-13E	39.0	5.4	8.9	4.4 (P)	39.2	5.4	7.9	4.4 (P)
Ic-15E	37.0	5.0	7.3	4.2 (P)	36.7	5.1	7.5	4.1 (P)
Ic-15P	28.1	3.5			28.3	3.5		

Representative alcohols of Formula (I) (listed in Table 1a) show selective cytotoxicity towards human cancer cell lines transfected with either the *E. coli* nitroreductase cDNA (NTR) (Table 3, columns 2 and 3), or human cytochrome P450 reductase (P450R) under hypoxic conditions (Table 3, columns 4 and 5). In this table, sensitivity ratios are
5 displayed to indicate the degree of selectivity for either NTR expression (column 3) or hypoxia (column 5). However, overexpression of P450R is not required for hypoxic selectivity.

IC₅₀ values are derived from cell proliferation experiments, following 4 hour drug
10 exposure under a gas phase of either 20% oxygen or 0% oxygen (anoxia, achieved using an anaerobic chamber). Cells were grown under aerobic conditions for a further 5 days, and cell density estimated using the sulphorhodamine B assay, to determine the concentration of prodrug required to inhibit growth to 50% of control.

Table 3. Selective cytotoxicities of representative examples of the alcohols of Table 1a

No	Human colon (4 h)		Human lung (4 h)	
	WiDr (NTR ^{+ve}) IC ₅₀ (μM)	WiDr WT:NTR IC ₅₀ Ratio	A549 (P450R ^{+ve}) anoxia IC ₅₀ (μM)	A549 (P450R ^{+ve}) 20% O ₂ /anoxia IC ₅₀ Ratio
IIa-1	5.2	34	3.7	28
IIa-2	48	26	25	3.7
IIa-3	47	36	54	23
IIa-7	1.5	99	6.7	49
IIa-7s	9.3	35	2.1	109
IIa-8	1.6	224	23	6.6
IIa-9	6.4	58	22	9.4
IIa-10	10	22	-	-
IIa-11	11	9	-	-
IIa-12	4.2	116	73	10
IIa-13	5	90	32	18
IIa-14	2.9	49	13	4.5
IIb-1	61	2	384	<1.3
IIb-2	11.8	47	18	20
IIb-3	13.6	59	30	9
IIb-4	14	18	-	-
IIb-5	13	19	-	-
IIb-6	27	5	-	-
IIb-7	0.3	61	0.8	56
IIb-7a	0.5	27	1.0	5.3
IIb-8	0.4	13	1.1	24
IIb-9	0.9	5	1.4	20
IIb-10	0.9	2	2.3	11
IIb-11	1.0	2	6.6	4.5
IIb-12	0.4	48	0.28	133
IIb-13	0.3	27	0.15	138
IIb-14	0.8	12	1.0	27
IIb-15	0.3	31	0.28	118
IIc-7	10	46	3.9	40
IIc-8	5.0	70	6.6	24
IIc-9	31	6	7.3	21
IIc-12	5.0	84	2.6	173
IIc-13	4.3	95	4.5	134
IIc-14	20	16	7.1	57

The activity of the phosphates as hypoxic cytotoxins is demonstrated by the data in Figure 1 for the representative example (**Ib-7P**). This employs an *in vivo* excision assay with the Rif-1 tumour, where the oxic tumour cells are sterilised using 15Gy of radiation, and the cytotoxicity of an agent against the remaining hypoxic cells can be quantitated.

- 5 Unexpectedly, the activity of the phosphate **Ib-7P** is found to exceed that of its parent alcohol (**Ib-7**) at their respective maximum tolerated doses (**Ib-7P** = 750 $\mu\text{mol/kg}$; **Ib-7** = 1000 $\mu\text{mol/kg}$). This experiment demonstrates that the phosphate **Ib-7P** is more active against hypoxic cells than the reference hypoxic cytotoxin tirapazamine, and that it is more active against hypoxic cells than oxic cells (i.e. when given post irradiation than
- 10 when given without irradiation). Thus **Ib-7P** acts as a hypoxia-selective cytotoxin *in vivo*. Although less active against oxic tumour cells, this activity is significant demonstrating that the compound also has utility as a single agent (without radiation).

Figure 1. *In vivo* activity of **IIb-7** and its corresponding phosphate pre-prodrug **Ib-7P**, relative to the known hypoxic cytotoxin tirapazamine.

Figure 1a.

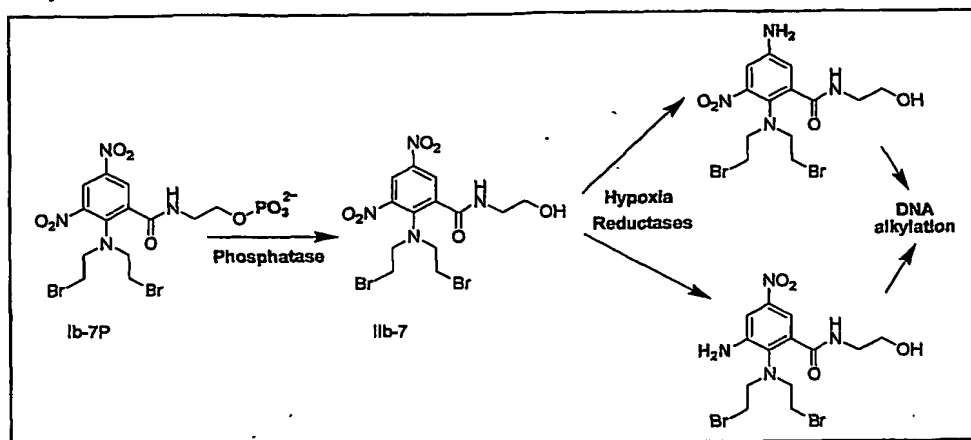
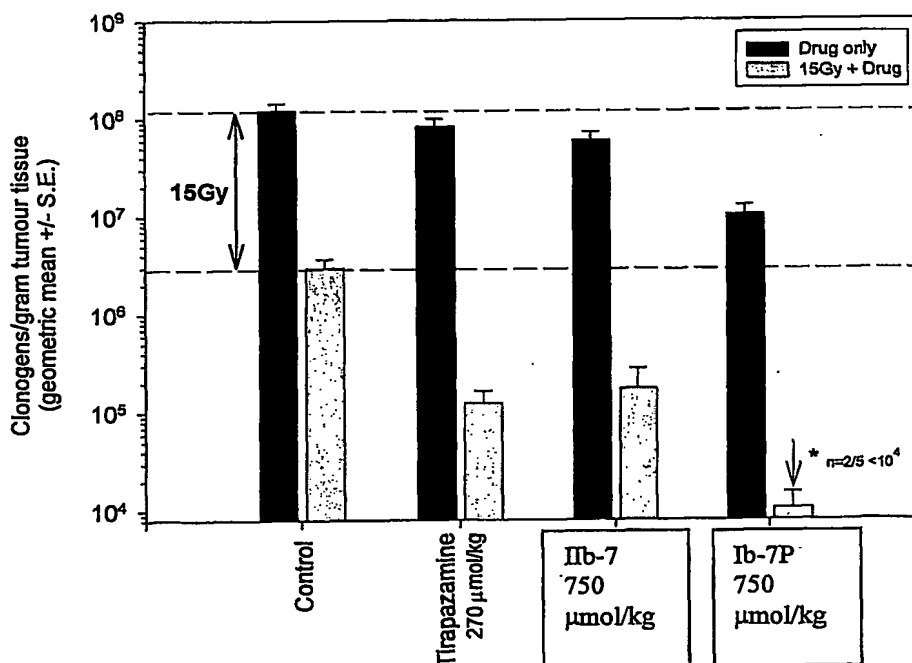


Figure 1b.

Excision assay method: 300-500mg Rif-1 xenograft → 15Gy → Single prodrug dose @ MTD → 18hr → excision → disaggregate → plate 10^2 - 10^5 cells → 12 days → colonies counted.



The notable activity of the phosphates of Formula (I) against hypoxic cells in human tumour xenografts is illustrated by the data of Table 4. In these experiments SiHa human cervical carcinoma cells were grown subcutaneously in CD-1 nude (immunodeficient) mice. The compounds were administered at doses corresponding to 75% or 20% of the maximum tolerated dose (MTD), 5 minutes after a whole body dose of ionising radiation (cobalt-60 gamma radiation) sufficient to sterilise the oxic cells (15 Gy). The tumours were excised 18 hours later, dissociated with a cocktail of proteases, and cell survival was determined using a clonogenic assay. The logarithms of cell kill were calculated from the difference in the numbers of clonogens per gram tumour tissue between treated and control tumours. All of the phosphates tested showed large effects against hypoxic cells at 75% of the MTD (Table 4, column 4). This was selective for hypoxia as demonstrated by the lesser cell kill in the absence of radiation. However, cell killing by the compounds alone was significant in all cases (Table 4, column 5) demonstrating that the compounds also have antitumour activity as single agents. Activity against hypoxic cells was also demonstrated for the same compounds at doses corresponding to only 20% of the MTD (Table 4, column 7). The reference hypoxic cytotoxin tirapazamine, and the reference nitrogen mustards (melphalan, chlorambucil and cyclophosphamide) lacked activity at 20% of their respective MTDs.

Table 4. Activity of phosphates of Formula (I) against oxic and hypoxic cells in SiHa human tumour xenografts in nude mice. Compounds were administered as single i.p. doses in saline.

No	MTD ($\mu\text{mol/kg}$)	75% of MTD			20% of MTD	
		Dose ($\mu\text{mol/kg}$)	Log kill, cmpd only ^a Mean \pm sem	Log kill, cmpd after radiation ^b	Dose ($\mu\text{mol/kg}$)	Log kill, cmpd after radiation ^b
Ia-8P	1000	750	ND ^c	ND	200	-0.01 \pm 0.19
Ib-2P	237	178	ND	ND	47	-0.10 \pm 0.08
Ib-2mP	1780	1330	2.47 \pm 0.08	\geq 3.47 \pm 0.17	356	2.56 \pm 0.37
Ib-7P	750	562	1.20 \pm 0.07	2.35 \pm 0.17	150	1.12 \pm 0.13
Ib-12P	1330	1000	1.32 \pm 0.30	\geq 3.38 \pm 0.28	267	1.65 \pm 0.19
Ib-14P	562	422	0.92 \pm 0.06	1.93 \pm 0.21	113	0.64 \pm 0.11

Ib-15P	1330	1000	2.24 ±0.15	≥ 3.27 ±0.13	267	1.74 ±0.23
Ic-6P	3160	2370	1.11 ±0.20	≥ 2.62 ±0.17	632	1.64 ±0.30
Ic-8P	562	422	ND	ND	113	0.72 ±0.08
Ic-12P	1780	1330	1.04 ±0.29	≥ 2.96 ±0.31	356	2.03 ±0.25
Ic-13P	3160	2370	2.36 ±0.28	≥ 3.23 ±0.08	632	1.60 ±0.20
Ic-15P	1780	1333	2.27 ±0.17	2.80 ±0.40	356	1.81 ±0.23
Tirapazamine	316	237	-0.02 ±0.01	0.66 ±0.11	63	-0.01 ±0.09
Chlorambucil	237	178	0.11 ±0.04	0.31 ±0.10	47	0.18 ±0.13
Melphalan	42.2	31.6	ND	ND	8.4	0.04 ±0.05
Cyclophosphamide	750	562	ND	ND	150	0.07 ±0.10

^a Relative to controls, in the same experiment, treated with vehicle (saline) only. Log kill =

$\log_{10}(\text{clonogens/g tumour for control tumours}) - \log_{10}(\text{clonogens/g tumour for treated tumours})$.

^b Relative to the mean for radiation only, in the same experiment. Log kill = $\log_{10}(\text{clonogens/g tumour for radiation alone}) - \log_{10}(\text{clonogens/g tumour for tumours treated with radiation plus compound})$.

5 ^c None detected

A representative example of the phosphates of Formula (I) as NTR activated cytotoxins is provided in Figure 2. In the WiDr *in vivo* growth delay assay, xenografts containing mixtures of WiDr^{WT} and WiDr^{NTR} cells are grown to 300mm³ and treated with a single
10 dose of prodrug at its MTD. Tumour growth is monitored over time and animals are euthanased when mean tumour volume >1600mm³. Data is presented as time to death. Unexpectedly, the activity of the phosphate (Ib-7P) is observed to exceed that of its parent alcohol (Ib-7), when administered at their respective maximum tolerated doses (750 µmol/kg). Ib-7P is superior to Ib-7 with respect to (i) time to first treatment failure (77-
15 days vs. 17-days) and (ii) over-all survival (40% vs. 6%).

Figure 2. *In vivo* activity of IIb-7 and its corresponding phosphate pre-prodrug, Ib-7P, against human colon carcinoma xenografts grown by inoculating mixtures of 90% NTR – ve and 10% NTR+ve Wi Dr cells.

Figure 2a.

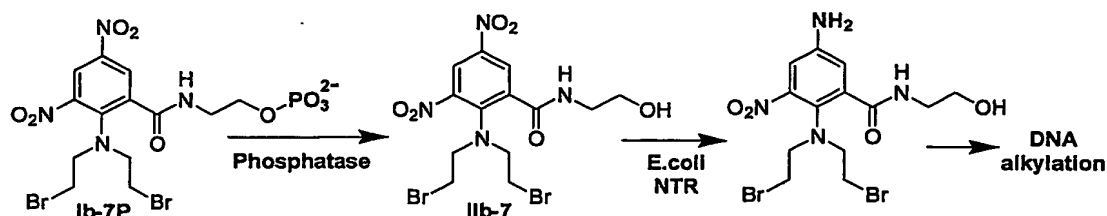
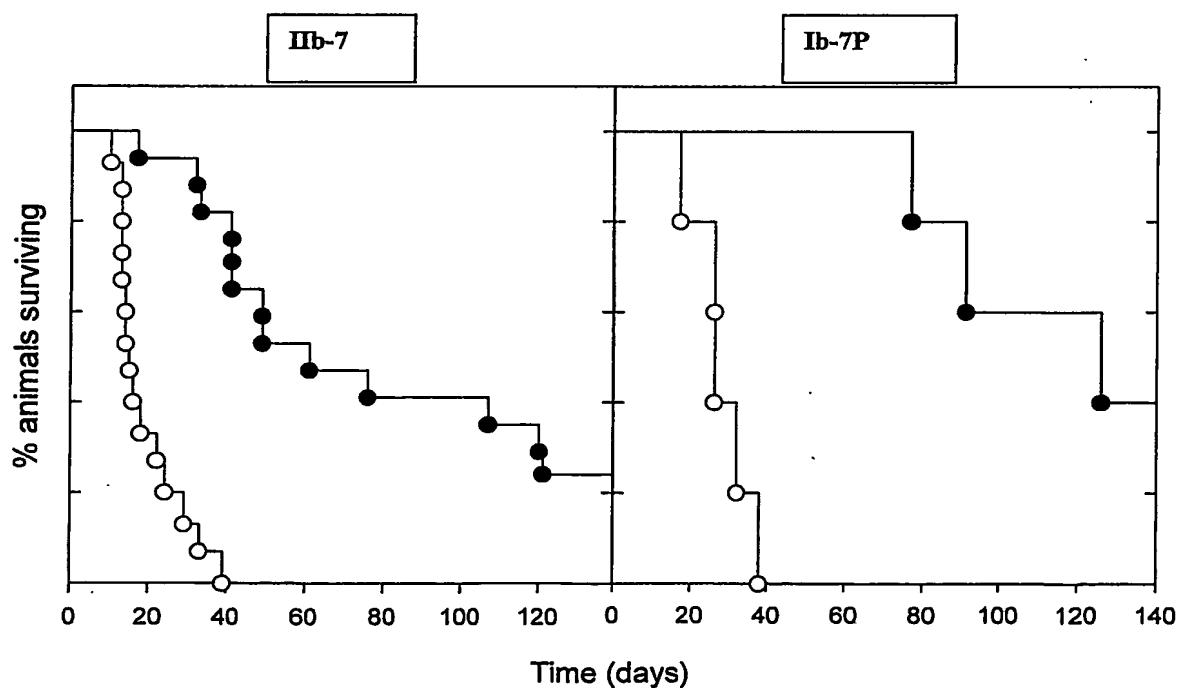


Figure 2b.



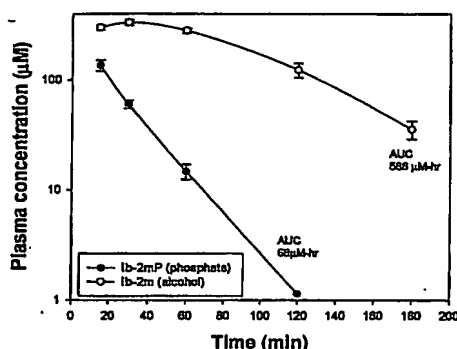
Legend: Open circles: wild-type WiDR human colon carcinoma xenografts

Filled circles: Tumours containing WiDr cells transfected with the *E. coli* NTR gene.

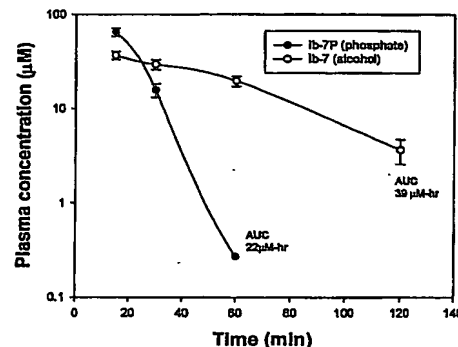
Pharmacokinetics of the phosphate esters Ib-2mP, Ib-7P, Ib-12P and Ic-12P following administration to female CD-1 nude mice by intraperitoneal injection at a dose corresponding to 75% of the maximum tolerated dose. Monosodium salts of the compounds were dissolved in phosphate buffered saline, pH 7.4, with addition of one equivalent of sodium bicarbonate. Serial blood samples were obtained by small tail vein bleeds, and 10 μ l of plasma was prepared from each. Proteins were precipitated by addition of 3 volumes of methanol, and concentrations of the phosphate esters and corresponding alcohols were determined by HPLC using either UV or mass spectrometry detection.

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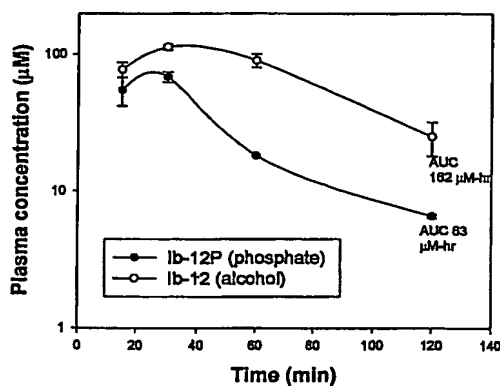
Cmpd Ib-2mP (1.333 mmol/kg, i.p., female CD-1 nude mice)



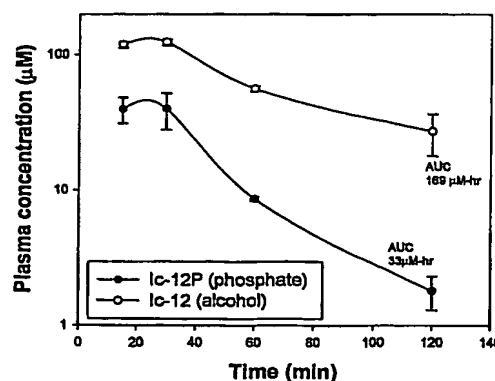
Cmpd Ib-7P (0.562 mmol/kg, i.p., female CD-1 nude mice)



Cmpd Ib-12P (1 mmol/kg, i.p., female CD-1 nude mice)



Cmpd Ic-12P (1.33 mmol/kg, i.p., female CD-1 nude mice)



The data shows that the phosphate esters are converted efficiently to the corresponding alcohols in mice. The alcohols are the species activated by nitroreduction in hypoxic, or NTR-expressing, cells.

Wherein the foregoing description reference has been made to reagents, or integers having known equivalents thereof, then those equivalents are herein incorporated as if individually set forth.

- 5 While this invention has been described with reference to certain embodiments and examples, it is to be appreciated that further modifications and variations may be made to embodiments and examples without departing from the scope of the invention.